

# **BOOK OF PROCEEDINGS**

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### Foreword

The current agro-food system failed to feed the world population and to eradicate rural poverty. The environmental and social problems caused by such an agricultural production model are well known and widespread. The most dramatic environmental damage is caused by climate change, loss of biodiversity and ecosystem functions, soil erosion and degradation, and pollution from fertilizers and pesticides.

Climate change, population growth and competing demands for land and resources are putting great pressure on the world's food systems. The world's population is set to reach nine billion by 2050. To feed them it is needed to produce 70% more food, and do so without destroying our environment. The successful management of agricultural resources for meeting the changing human needs, while maintaining or enhancing the quality of the environment and conserving natural resources, is imperative for a stable food production.

Agriculture is at the core of sustainable development. Ecological agriculture, agro-ecological practices and sustainable agricultural production are alternative farming methods to address the environmental consequences of conventional agriculture dependent on intensive chemical inputs. Sustainable agriculture is not only a technological solution but also an approach that embodies a shift in agricultural paradigms. Sustainable food systems should integrate the economic, social and environmental dimensions of sustainable development.

Appropriate agricultural and rural development strategies in the Balkan area and beyond should ensure food and nutrition security - through the sustainable and eco-functional intensification of crop and animal production systems - while conserving the natural resource base. They should also contribute to the eradication of rural poverty and improvement of livelihoods and quality of life of rural populations.

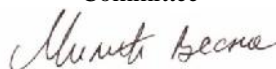
Innovative and responsible measures - based on sound and accurate scientific knowledge - are required to respond to the multiple food-related challenges of the New Millennium in a resource-constrained world. During four days; October 3-6, 2013; the 4<sup>th</sup> International Symposium “Agrosym 2013” made an important contribution to the improvement of knowledge in agriculture, environment and rural development fields. In fact, 224 papers were presented by scientists from 38 countries (Albania, Algeria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Egypt, Finland, Germany, Ghana, Greece, India, Indonesia, Iran, Iraq, Italy, Japan, Kazakhstan, Lebanon, Republic of Macedonia, Malaysia, Montenegro, Morocco, Niger, Pakistan, Poland, Romania, Russia, Serbia, Slovenia, South Africa, Spain, Slovakia, Tunisia, Turkey, Ukraine, Vietnam). This publication comprises all accepted full papers.

The success of the symposium was made possible thanks to the unconditional commitment and invaluable contributions of a wide range of partners and sponsors. Much appreciation is due to the authors of all papers submitted to and presented at the symposium, the reviewers for their sound comments and feedback as well as to all the symposium participants for ideas, insights and contributions.

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**KEYNOTE PAPERS**

## NEW TECHNOLOGIES FOR IMPROVING MAIZE BREEDING

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### Abstract

Limited sources of germplasm used in maize breeding programs, increased the importance of prebreeding activities. Advances in technologies have allowed other methods to add efficiencies to modern commercial maize breeding. These include the use of Doubled haploids, genomics, molecular markers and transformation. Doubled haploids method creates completely homozygous inbreds in 1-2 years versus 7 in traditional breeding. Molecular markers serve as a starting point for genes and have a significant role in selection. MAS is the use of markers to identify the presence of a specific gene or combination of genes that carry a desirable trait, which allows direct use of the inbred to create specific combinations and more rapid trait improvements can be made. Genomics helps scientists to identify which genes determine important traits, and how genes interact with each other. The complete DNA sequence of the maize genome, along with more comprehensive transcriptome, proteome and metabolome information, help to further unravel the complexities of how genes and gene networks function to produce productive maize plants. Genetic improvement is resulting in improved agronomic, disease, and/or end use traits. Application of all new technologies will help breeders to achieve greater harvestable yield and product development systems.

**Key words:** maize, prebreeding, molecular breeding, genomics

### Introduction

Maize is an important crop for food, feed, forage, and fuel across tropical and temperate areas of the world. It is also a classical genetic model for plant research. It has a number of characteristics that are favorable for an experimental model for crop plants: a multiple purpose crop with worldwide cultivation; <59 000 and 42 000–56 000 genes with moderate genome size (~2400Mb of DNA per haploid nucleus in the B73 inbred), which is approximately six times larger than rice and six times smaller than wheat, although a large proportion of the genome represented by repetitive elements; outbreeding reproduction system with tolerance of inbreeding; existence of multiple breeding products (inbreds, hybrids, synthetic cultivars, open-pollinated varieties improved landraces), and wide adaptability including good sources of resistance to environment stresses. The maize genome harbors tremendous molecular diversity that mirrors its substantial phenotypic variability. When considering nucleotide polymorphism in genes, two maize lines are on average as diverged from one another as humans are from chimpanzees (Buckler et al, 2006).

The conventional breeding is the mainstay of inbred and hybrid development. It is based on the theory of heterosis. Advances in technologies have allowed other methods to add efficiencies to modern commercial maize breeding (Babic et al 2011). These include the use of doubled haploids, genomics, molecular markers and transformation. These technologies have allowed scientists to make more informed decisions around specific genetic combinations to improve genetic gain, and allowed for more rapid identification of lines carrying a particular trait of interest. Utilization of conventional breeding coupled with new technologies has led to the rapid increase in yield gain in maize.

Doubled Haploid (DH) lines are routinely applied in much commercial hybrid maize breeding programs. Doubled haploids are created by a special genetic process and have one set of chromosomes. They undergo chromosome doubling through a chemical process that produces a completely homozygous, fertile doubled haploid plant. Conventional inbred development, it takes seven generations to do this, and the plants are still not 100 percent pure. The purity and genetic uniformity of doubled haploid lines make it easier to measure characteristics and reduce product development time.

Considering the progress in the various “omics” areas and the integration of different disciplinary applications facilitated by bioinformatics, as well as high-throughput genotyping approaches combined with automation, MAS will gradually evolve into more holistic “genomics-assisted” breeding strategies. Since genomics resources in maize are among the best of the major crop species, the role of genomics is set to become more and more important in maize breeding. However, conventional selection will remain a vital element of the process to finally confirm the best candidate genotypes for progression into the advanced stages of crop improvement and cultivar selection. MAS will not replace conventional breeding programs, but rather will increase reliance on genomics data alongside other technology interventions in an ever evolving and refining breeding system. Genomics-assisted breeding systems will be evaluated in terms of their ability to increase the scope of breeding goals, to provide new added-value traits, to decrease the cost of breeding programs, and to improve the pace of developing new cultivars, and finally to enhance impact of resultant products to command increasing areas of production.

#### Prebreeding

Prebreeding concept represent a link between genetic resources and breeding activities, aimed to develop germplasm directly or indirectly usable for creation of new cultivars (Nass and Paterniani, 2000). Prebreeding programs can develop new base populations and also help in heterotic patterns identification, necessary for maize breeding. An additional advantage is the establishment of core collections. Maize Research Institute (MRI) Zemun Polje has a genbank which is among the ten largest in the world (FAO, 2010). It conserves collection of 2217 landraces from the former Yugoslav territories and 3258 introduced genotypes from 40 countries (Andjelkovi and Ignjatovi -Mici , 2012). Local landraces are not used directly in breeding, rather for the creation of synthetic populations or for the formation of core collections. In the last decade different research programs had aim to identify superior genotypes within accessions at MRI gene bank. In order to identify cytoplasm types sources of CMS were screened using PCR assay with specific primers for C, T and S cytoplasm (Van etovi et al., 2010). A set of 54 landraces representing 18 agroecological groups from MRI collection was analysed for grain quality components (Mladenovic Drinic et al., 2011). Project of identification of new sources of drought tolerance among more than 6000 samples (gene bank accessions and elite inbred lines) started in 2008. All samples were grown under controlled drought stress in Egypt (Babic et al., 2011). A total of 672 accessions were chosen for further testing in temperate climate. After testing of general combining ability and heterotic pattern 41 genotype is selected for core collection formation. Among them, six accessions produced superior crosses with three lines representing different heterotic groups (Andjelkovi and Ignjatovi -Mici , 2012). Within current four year project the whole collection will be analysed for grain quality. Genotypes with the highest macro and micro nutrient content will be tested in the field conditions. Identified genotypes with high grain quality and/or drought tolerance will be genotyped using SNPs in order to identify alleles for specific target traits which would be used in breeding programs and improve their efficiency.

### Double haploids

The most common method of inbred line development in conventional maize breeding is based on pedigree method, but recently a new method of breeding that creates inbred lines, doubled haploids was developed. Two key differences between the Pedigree and Doubled haploid (DH) methods are that DH inbreds are completely homozygous and developed in 1-2 years versus 6-8 generation to derive lines with 99% homozygosity by conventional breeding. Use of doubled haploid (DH) lines produced by in vivo induction of maternal haploids are routinely used in maize breeding. To induce maternal haploids, the donor plant is pollinated by a specific maize stock (line, single cross, or population) called inducer. The first recognized inducer line was the genetic strain Stock 6, with an haploid induction rate of up to 2.3% (Coe, 1959), which was subsequently improved by hybridization and further selection. Today, modern haploid inducing lines display high induction rates of 8 to 10% (Geiger & Gordillo, 2009). Beside regular F1 kernels, the pollination results in a certain proportion of kernels with a haploid maternal embryo and a regular triploid endosperm. Such kernels display a normal germination rate and lead to viable haploid seedlings. After artificial chromosome doubling the successfully treated seedlings are selfed leading to completely homozygous and homogeneous progenies (DH lines).

A key issue to apply the in vivo haploid induction approach on a commercial scale is an efficient screening system allowing the breeder to differentiate between kernels or seedlings generated by haploid induction and those resulting from regular fertilization. Haploid embryos can be selected based on morphological and physiological markers. The most efficient haploid identification marker is the ‘red crown’ or ‘navajo’ kernel trait encoded by the dominant mutant allele R1-nj of the ‘red color’ gene R1, which causes deep pigmentation of the aleurone layer in the crown region and scutellum (Geiger & Gordillo, 2009). In a haploid inducing cross, the marker should be homozygous recessive in the female parent and homozygous dominant in the pollinator inducer line. After pollination, kernels with a red aleurone crown containing a nonpigmented scutellum are visually selected from the hybrid kernel of regular fertilization with both aleurone and scutellum pigmented. Another cheap and fast haploid identification method was suggested by Rotarenko *et al* (2007), who observed that kernels with a haploid embryo have a significantly lower oil concentration than those with a diploid F1 embryo. This is due to the reduced size of haploid embryos when compared with diploid embryos. Inducers with an above-average oil concentration should be best suited for this approach. Major advantages of DH lines in hybrid breeding are: maximum genetic variance between lines for per se and testcross performance from the first generation; complete homozygosity; acceleration of inbred line development; perfect fulfillment of DUS criteria reduced expenses for selfing and maintenance breeding; greater efficiency and precision of selection especially when used in combination with molecular markers, and increased efficiency in MAS, gene introgression, and stacking genes in lines. Because DH technology offers a faster way to obtain completely homozygous lines, it can save significant time and resources for implementing genetic studies: establishment of DH mapping populations, improve the precision of genetic and mapping studies, analysis of linkage disequilibrium, analysis of haplotype/trait associations, accelerate gene pyramiding, evaluation, and conservation of genetic resources, extraction of individual gametes from heterozygous materials transforming them into DH lines.

### Applications of molecular markers in maize breeding

Molecular markers have a significant role in selection. They serve as a starting point for genes that are responsible for the phenotypic response. Different kinds of molecular markers exist, such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs) markers, amplified fragment length polymorphisms (AFLPs), microsatellites and single nucleotide polymorphisms (SNPs). They may differ in a variety of ways; such as their technical acquirements; the amount of time, money and labor needed; the number of genetic markers that can be detected throughout the genome; and the amount of genetic variation found at each marker in a given population. The information provided to the breeder by the markers varies depending on the type of marker system used. Each has its advantages and disadvantages and, in the future, other systems are likely to be developed.

In the past 25 years, maize breeders have tried different ways of using molecular markers to improve grain yield and other agronomic traits. Molecular markers have been extensively used in maize genetic diversity studies for: analysis of genotype frequencies for identification of deviations at individual loci and for characterization of molecular variation within or between populations, construction of “phylogenetic” trees and determination of heterotic groups (Drinic et al., 2011) and, analysis of correlation between genetic distance and hybrid performance, heterosis and specific combining ability (Srdic et al 2011, Drinic et al, 2012).

The development of single nucleotide polymorphism (SNP) markers in maize offers the opportunity to utilize DNA markers in many new areas of population genetics, gene discovery, plant breeding and germplasm identification. Among all types of markers, SNP markers are increasingly the marker-of-choice for all genomics applications in maize breeding. SNP markers use in various areas of molecular genetics and plant breeding, including gene/QTL mapping, linkage-disequilibrium-based association mapping, map-based gene/QTL cloning, germplasm characterization, genetic diagnostics, event characterization, marker-assisted trait introgression, and finally marker-assisted selection (MAS). In order to conduct most of the above-mentioned SNP applications, researcher must know the order of the markers on chromosomes, which can be obtained by constructing recombination-based genetic linkage maps. SNP discovery has been performed on over 3,000 genes, with genetic mapping data on over 1,100 SNP markers being collected on Nested Association Maps using diverse maize inbred lines (Yu et al. 2008). Compared to other types of molecular markers, SNP markers have several advantages, including high abundance and even distribution through the genome. In addition, SNP markers provide highly reproducible codominant information, and there is an increasing range of cost effective high-throughput SNP genotyping systems. Most of the public SNP markers in maize are developed from B73 and Mo17 cultivars. Taking into account massive intraspecific variations among maize inbred lines (Springer et al., 2009), SNPs developed from a few lines will capture only a small portion of all allelic variations happening between parents of a cross designed for a QTL study. Over 2000 SNP markers that were developed for SNP chip-based genotyping are being mapped using three RIL populations.

Molecular marker-facilitated mapping of genes underlying specific traits in maize was first reported by Stuber et al. (1987) followed by Edwards et al. (1992). Since then, more than 2000 QTL related to various traits of agronomic importance in maize, including yield, yield components, plant morphology and physiology, and biotic and abiotic stress responses have been reported (<http://www.maizegdb.org>). Genetic mapping has been developed through conventional linkage mapping and more recently through linkage disequilibrium-based association analyses. Genetic mapping in maize was first carried out using morphological markers generating a genetic map consisting of 62 morphological trait loci. The first generation of molecular marker maps in maize was constructed using RFLP, which were later

saturated with SSR and other types of PCR-based markers. Most recently, linkage mapping is being raised to a new level as maps are being developed with large numbers of SNP markers and/or candidate gene-based markers. Genetic mapping is carried out using segregating populations, including F<sub>2</sub>, backcross, recombinant inbred lines (RILs) or doubled haploids. In order to improve the resolution and extend the total map distance, the Maize Mapping Project developed RILs through several generations of intermating an F<sub>2</sub> population derived from the single cross of the inbred lines B73 and Mo17. As a result, the resolution of the genetic map was improved significantly, consisting of about 1000 RFLP and 1000 SSR markers.

Using the marker map, genes affecting traits of interest can then be detected by testing for statistical associations between marker variants and any trait of interest. These traits might be genetically controlled by one or a few genes. Alternatively, they could be genetically complex quantitative traits, involving many genes (i.e. so-called quantitative trait loci [QTL]) and environmental effects. Association mapping is an important tool to identify genes responsible for quantitative variation of complex traits by examining the marker-trait associations that can be attributed to the strength of linkage disequilibrium between markers and functional polymorphisms across a set of diverse germplasm (Zhu et al. 2008). Association mapping in plants can be based on candidate genes or whole genome scanning. The latter has become increasingly applicable in maize due to the recent development of large numbers of SNP markers. As a new alternative to traditional linkage analysis, association mapping offers three advantages, increased mapping resolution, reduced research time, and greater allele number (Yu and Buckler, 2006).

Marker-assisted selection (MAS) as a process refers to the selection of superior genotypes using molecular markers. In contrast to phenotypic selection, MAS does not rely on environmental conditions because it detects the structural polymorphisms at molecular level, requires leaf tissue collected at seedling stage, which is very useful for traits that are expressed at later stages of development and which also helps to avoid adverse weather conditions that could kill the plant at adult stage, could be cheaper and less labor intensive, allows selection in off-season nurseries and has a potential to accelerate breeding process. The essential requirements for MAS in breeding are: marker(s) should co-segregate or be closely linked (1 cM or less is probably sufficient for MAS) with the desired trait; an efficient means of screening large populations for the molecular marker(s) should be available. At present, this means, relatively easy analysis based on PCR technology; the screening technique should have high reproducibility across laboratories, be economical to use and be user friendly. According to Collard and Mackill (2008) applications of MAS in plant breeding were grouped into four broad categories: (1) marker-assisted germplasm evaluation including pedigree verification, purity assessment, evaluation of genetic diversity, identification of heterotic patterns and event characterization; (2) marker-assisted trait introgression, (3) marker-assisted pyramiding of genes and (4) genomic selection (GS). Current MAS strategies fit the breeding programs for traits with high heritability and are governed by a single gene or one major QTL that explains large portion of the phenotypic variability. However, the application of MAS for breeding traits with complex genetics based on the interaction of multiple QTL with minor effects has been inefficient. In classical MAS projects researchers use molecular markers that show statistically significant association with a phenotype and are linked to major QTL. Because minor QTL have small effects on phenotype, they have not been applicable in MAS. In comparison to conventional marker-assisted selection, which utilizes only a subset of genetic markers associated with a trait to predict breeding values (BVs), genome-wide selection (GWS) improves prediction accuracies by incorporating all markers into a model simultaneously. This strategy avoids risks of missing quantitative trait loci (QTL) with small effects. Bernardo and Yu (2007) compared

genome wide selection with marker-assisted recurrent selection by evaluating doubled haploids for testcross performance in Cycle 0, followed by two cycles of selection based on markers. They found that across different numbers of quantitative trait loci (20, 40, and 100) and levels of heritability, the response to genome wide selection was 18 to 43% larger than the response to MARS. Guo et al (2013) evaluated the accuracy of prediction for three corn flowering traits days to silking, days to anthesis, and anthesis-silking interval with GWS based on cross-validation experiments using a large data set of 25 nested association mapping populations in maize. GWS via ridge regression-best linear unbiased prediction (RR-BLUP) gave significantly higher predictions compared to MAS utilizing composite interval mapping.

#### “Omica” technologies

Genomics is a relatively new field of study, and may be described as the science of the genetic material of a chromosome set. This tool helps scientists identify which genes determine important traits in maize, and how genes interact with each other. Functional genomics utilizes the vast wealth of data produced by genome sequencing projects to understand the gene functions, and their interactions. It is often referred to the study of the genes, their functions, interactions, and regulation to provide a biological function in an organism. There are four major types of high-throughput measurements that are commonly performed: genomic SNP analysis (i.e., the large-scale genotyping of single nucleotide polymorphisms), transcriptomic measurements (i.e., the measurement of all gene expression values in a cell or tissue type simultaneously), proteomic measurements (i.e., the identification of all proteins present in a cell or tissue type), and metabolomic measurements (i.e., the identification and quantification of all metabolites present in a cell or tissue type). Each of these four is distinct and offers a different perspective on the processes underlying disease initiation and progression as well as on ways of predicting, preventing, or treating disease. Transcriptomic measurements (often referred to as gene expression microarrays or "gene chips") are the oldest and most established of the high-throughput methodologies. The most common are commercially produced "oligonucleotide arrays", which have hundreds of thousands of small (25 bases) probes, between 11 and 20 per gene. RNA that has been extracted from cells is then hybridized to the chip, and the expression level of ~30,000 different mRNAs can be assessed simultaneously.

#### Maize genome sequencing

Gene sequencing technology is used to determine the nucleotide sequence of genes or regions of the genome important for trait improvement. This technology has also been used to create a dense genetic map that helps to locate a specific gene or trait, detect the amount of genetic diversity that exists for that specific gene or trait, and to develop DNA-based diagnostic markers for the trait for use in the breeding programs. Gene sequencing makes trait selection much more accurate, and help breeders to improve plant breeding and product development systems. This allows more rapid creation and testing of superior products by selecting the best possible traits for yield and disease resistance. The application of gene sequencing technology to native trait discovery and molecular breeding is in active area of use.

Sanger sequencing method dominated the industry for almost two decades and still considered the gold standard for sequencing, but its limitations, especially with respect to throughput and cost, necessitated high demand for new and improved technologies for sequencing genomes. These alternative technologies collectively termed as Next generation sequencing (NGS) technologies (Varshney et al., 2009).

A wide range of sequence level variation exists in maize including single nucleotide polymorphisms (SNPs), small insertions/deletions, presence/absence variation (PAV), and



copy number variation (CNV). The US-based consortium of researchers decoded the genome of an inbred line of maize B73, an important commercial crop variety. The 2.3-billion-base sequence includes more than 32,000 protein-coding genes spread across maize's 10 chromosomes. Transposable elements are the most abundant parts of the sequence, spanning almost 85% of the genome (Schnable et al. 2009). A maize variety from the Mexican highlands called Palomero were sequenced (Viella-Calzada,2009) and the Palomero genome is around 400 million nucleotides smaller and contains about 20% less repetitive DNA than B73. More than a dozen genes related to heavy-metal detoxification and environmental-stress tolerance that were conserved in B73 and Palomero were found, but that were absent from teosinte, suggesting that these genes were involved in the domestication process. Another team, from Cornell University in Ithaca, sequenced part of the gene-rich region of 27 maize varieties to map haplotypes. This 'HapMap' revealed thousands of genes around the centres of the chromosomes, where they were unlikely to be shuffled around during recombination. Recombination is necessary for plant breeders to unite favourable genes from different crop varieties in a single plant, so this could explain why farmers often need to cross-breed different inbred lines to produce the superior maize varieties. Schnable et al (2009) compared the genome structures of B73 with inbred line Mo17. They found hundreds of genes that appeared only once in one or other of the two genomes. This suggests that crossing the two varieties could produce hybrids containing a higher number of beneficial genes.

Most of the public SNP markers in maize are developed from B73 and Mo17 cultivars. Consequently, there is a big chance that majority of allelic variations, including the causative mutation between parents of this cross will be missing. In order to avoid this situation, resequencing of genomes of both parents and discovery of allelic variations in low and single copy regions could be implemented using NGS technologies coupled with genome complexity reduction techniques. Discovered cross-specific polymorphisms can later be converted into any modern SNP genotyping assay (Mammadov et al., 2010).

RNA based sequencing (RNA-seq) is a powerful approach for transcriptional analysis, assessing sequence variation, and identifying novel transcript sequences, particularly in large, complex, repetitive genomes such as maize. Hansey et al (2012) sequenced RNA from whole seedlings of 21 maize inbred lines representing diverse North American and exotic germplasm. *De novo* assembly of RNA-seq reads that did not map to the reference B73 genome sequence revealed 1,321 high confidence novel transcripts, of which, 564 loci were present in all 21 lines, including B73, and 757 loci were restricted to a subset of the lines. 145 of the novel *de novo* assembled loci were present in lines from only one of the two heterotic groups consistent with the hypothesis that, in addition to sequence polymorphisms and transcript abundance, transcript presence/absence variation is present and, thereby, may be a mechanism contributing to the genetic basis of heterosis.

The maize sequencing project and the constant progress in maize functional genomics are providing new genes and functional genomic DNA sequence information that are increasingly being integrated into the maize genetic map. A total of 25 908 markers have now been integrated into the fingerprinted BAC contig (FPC) map. This includes 1902 genetically mapped markers (SSRs, RFLPs, SNPs, and InDels) and 24 006 sequence-based markers (ESTs, BAC ends, and 40-bp overlapping oligonucleotide overgo probes) (Cone et al. 2009).

#### Maize transformation

In maize transformation has been extensively used for the development of new commercial pest and herbicide resistant cultivars but more recently also including more complex traits such as grain quality and drought tolerance. Transgenic maize has been cultivated commercially in the United States since 1996. Since then, GM maize production has

expanded to more than 51 million hectares (32%) worldwide. Two traits are expressed by today's GM maize cultivars: insect resistance and herbicide tolerance. More and more, cultivars are being grown that express both of these traits simultaneously (stacked genes). The commercial sector has made substantial progress with pest resistant maize through transformation with genes encoding for insecticidal crystal (Cry) proteins from *Bacillus thuringiensis* (Bt), which have been particularly successful in providing protection against several corn borers. *Bacillus thuringiensis* is a species of bacteria that produces proteins that are toxic to certain insects. There are a number of Cry toxins that are categorized by their spectrum of activity. For maize pests, primary Cry proteins are Cry1 and Cry2 for Lepidoptera and Cry3 proteins for Coleoptera. Maize can be genetically engineered to produce these specific Cry toxins. IMI (IR/IT) or clearfield (CL) maize was developed by tolerance selection to be resistant/tolerant to imidazolinone herbicides. LibertyLink maize is genetically engineered to allow over-the-top applications of glufosinate herbicide and Roundup Ready maize allows postemergence applications of Roundup and some other glyphosate-type products directly to maize. Naqvi et al. (2009) created elite inbred South African transgenic corn plants in which the levels of three vitamins were increased specifically in the endosperm through simultaneous modification of three separate metabolic pathways. The kernels of the transgenic white corn (Cv. M37W) were found to contain 169-fold the normal amount of carotene, 6-fold the normal amount of ascorbate, and double the normal amount of folate. SmartStax is genetically modified (GM) maize that has eight GM traits combined or 'stacked' together, six for insect resistance (Bt) and two for herbicide tolerance. The traits are combined together using crosses between existing transgenic corn lines rather than using genetic transformation of a single maize strain. While the adoption of first generation traits in maize has been rapid, the next generation, currently in development, holds even more promise. These traits are designed to help maize continue to grow under drought conditions, more efficiently use nitrogen, produce even higher yields, enhance protection against insects and other pests, and improve grain quality for food, animal feed and biofuels.

### Conclusion

New molecular technology including molecular markers, genomics, gene sequencing, and transformation has been widely used in maize breeding. It covers many different applications that influence the understanding of gene function. Application of these technologies does not occur independently of conventional breeding, but in association with it. A number of breeding programs have in the past two decades to varying degrees started using markers to increase the effectiveness and to significantly shorten the development time of varieties and therefore maize geneticist consider molecular marker assisted selection a useful additional tool in breeding programs to make selection more efficient. The most significant breakthrough in agricultural biotechnology is coming from research into the structure of genomes and the genetic mechanisms behind economically important traits. The genomics provide information on the identity, location, impact and function of genes affecting important traits, cataloging and mapping single gene markers. The complete DNA sequence of the maize genome, along with more comprehensive transcriptome, proteome and metabolome information, will continue to drive innovations in molecular breeding and biotechnology. These additional layers of information help to further unravel the complexities of how genes and gene networks function to produce productive maize plants. This knowledge will lead to improved predictions and capabilities to assemble native gene variation through molecular breeding as well as more optimal gene selection and regulation in the development of future biotechnology products.

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**NEGATIVE EFFECTS OF IRON CHLOROSIS**

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**Abstract**

Three different experiments were conducted to determine the distribution and causes of iron chlorosis at Strategy I (grape vine) and Strategy II plant (maize). In first experiment, during 2007 field research on maize covered 132 sites. Chlorosis was quantified by visual ratings and chlorophyll meter readings. From the chlorophyll meter data collection the relative chlorosis (Kl\_rel) were calculated and mapped. Relative chlorosis has averaged 41.42% and increased with increasing pH value of the soil. Second experiment included vegetation pots trial in greenhouse. Maize hybrid OsSK 617 was sown in pots filled by regosoil, with two levels of field water capacity. Iron chlorosis was determined by chlorophyll meter readings, leaf chlorophyll concentration and leaf total Fe. Higher relative chlorosis (43.88%) was recorded in maize grown in wet condition as result of reduced synthesis of chloroplast pigments. Highly significant negative correlation was found between the relative chlorosis and total concentration of chloroplast pigments. The difference in the concentration of iron in the shoots of maize in dry and wet conditions was not statistically justified. Third research was conducted during 2009 on grape vine included field measurements of the intensity of chlorosis and laboratory analysis of soil and plant material. On soils with a high pH values and  $\text{HCO}_3^-$ , chlorosis was detected frequently, which is a common phenomenon that occurs as iron deficiency. The average value of the relative chlorosis was 36.45%. The concentration of iron in the leaves and petioles of grape vines was not significantly correlated with the relative chlorosis, which can be result of physiological inactivation of iron

**Key words:** maize, grape vine, iron chlorosis, chlorophyll meter

**Intoduction**

Iron deficiency chlorosis is one of the major abiotic stresses for plants grown on calcareous and/or alkaline soils because of an extremely low solubility of Fe (Mengel, 1994). Calcareous soils are characterized by high soil pH (between pH 7 and 8.5) (Loeppert and Hallmark 1985). Generally, the total Fe in soils is higher than the soluble Fe required for optimal growth, which is at approximately  $10^{-8}$  mol dm<sup>-3</sup> in the soil solution (Lindsay 1995). The availability of Fe<sup>3+</sup> in soils is low; it depends largely on pH and on redox potential and increases with low soil pH and low redox potential. The availability of free Fe<sup>3+</sup> is higher than in calcareous soil, but even in acidic soil it is usually too low to meet the plant demand for Fe (Lindsay and Schwab 1982). In calcareous soils, the concentration of Fe<sup>3+</sup> is very low (about  $10^{-10}$  mol dm<sup>-3</sup>; Lindsay 1995) and thus far too low to sustain optimal plant growth.

There are many factors that contribute to chlorosis including genetics, light intensity, ion imbalances, oxygen level, as well as nitrogen, magnesium, manganese, copper, and zinc availability. Iron deficiency induced chlorosis often appears as yellowing along the leaf margin and between the leaf veins. Since the veins themselves typically remain green, this

condition is referred to as “interveinal chlorosis.” Iron chlorosis appears on the youngest leaves because the element is very immobile within the plant, and is not translocated from older tissue as needed. Iron is a component of several substances that play key roles in plant physiology. One very important role is in the synthesis and degradation of chlorophyll, although iron itself is not a constituent of the chlorophyll molecule. Chlorophyll in turn plays an important role in plant carbohydrate metabolism (photosynthesis). When iron is lacking in the soil or is in an insoluble form, chlorosis may result. Water availability and temperature regime also influence on chlorophyll production and chlorosis. In other cases, root injury by soil tillage or even drought may cause decreased iron uptake and chlorosis. Sandy soils low in organic matter may also be iron deficient. Iron chlorosis can be identified by visual symptoms (several authors proposed the use of visual scores, from 0 -without symptoms, to 5 -very strong appearance of chlorosis, when more than 10% of leaves chlorotic), by the evaluation of chlorophyll content using chlorophyll meter – indirect method and by plant analysis – direct method. Depending on plants response to iron deficiency, plants can be grouped into Strategy I and Strategy II plants (Römheld and Marschner, 1986). Strategy I plants (dicotyledons and non graminaceous monocotyledons) respond to Fe deficiency by release of reductants - caffeic acid, increased  $H^+$  release, increased transport of Fe to tops and enhancing ferric reduction activity at the root plasma membrane (Chaney et al, 1972, Marschner, 1995). Strategy II plants synthesize and secrete non-proteinogenic amino acids - phytosiderophore. Iron is transported across the plasma membrane as  $Fe^{3+}$  phytosiderophore. Due to the different mechanisms of Fe acquisition, Strategy I and Strategy II plants have a different reaction on iron deficiency stress. The aim of this work was to determine the distribution and the causes for the appearance of chlorosis at Strategy I (grape vine) and Strategy II plant (maize).

### Materials and methods

#### Field research - maize

Field research was conducted during 2007 (Jug et al., 2008) on maize crops in the eastern Croatian, and included determination of the crop positions by the GPS, visual estimation of the crop chlorosis (Table 1) and determination of the total concentration of the chloroplast pigments at the chlorotic and nonchlorotic maize plants by indirect method - chlorophyll meter (SPAD, Minolta, Japan). From the chlorophyll meter data collection the relative chlorosis (Kl\_rel) were calculated (Formula 1) and mapped at the satellite maps (SRTM - *Shuttle Radar Topography Map*). All data were statistically analyzed by the usual methods.

Table 1. Visual estimation of the crop chlorosis

Fe chlorosis (%)	visual scores
no symptoms	0
less than 20% plants with symptoms	1
20-40% plants with symptoms	2
40-60% plants with symptoms	3
60-80% plants with symptoms	4
more than 80% plants with symptoms	5

Formula 1

$$Kl_{rel} = 100 - \left( \frac{Kl_S}{Kl_N} \times 100 \right)$$

$Kl_{rel}$  = relative chlorosis (%)

$Kl_S$  = SPAD value – leaves with chlorosis symptoms

$Kl_N$  = SPAD value – leaves without chlorosis symptoms

#### Vegetation trial in the pots – maize

The vegetation pots trial has been conducted in the greenhouse. The 40 dm<sup>3</sup> large pots were filled by the regosoil, by maintaining natural stratification of the soil horizons (A-C). Experiment included two levels of field water capacity (50% FWC and 100% FWC) simulating drought and excessively wet conditions. The used maize hybrid was OsSK 617. In the 7-8 leaves stage maize plant material has been collected and analyzed: readings by chlorophyll meter; chlorophyll concentration was determined spectro-photometrically (at wave lengths 662, 644 and 440 nm) from an acetone extract using the methods of Holm and Wettstain and expressed in mg per g of fresh mass (Arsenijevi -Maksimovi and Pajevi, 2002) and plant materials were destruction by wet digestion (mixture of sulfuric and perchloric acid and hydrogen peroxide), (Vukadinovic and Berti, 1988.). Concentration of the iron was determined directly from the stock solution by atomic absorption spectrophotometry (AAS). All data were statistically analyzed by the usual methods.

#### Field research - vineyards

Research was conducted during the grape vine growing season 2009 in the full floral and early ripening grape, in the area of Baranja and Erdut vineyards (Jug et al., 2012).

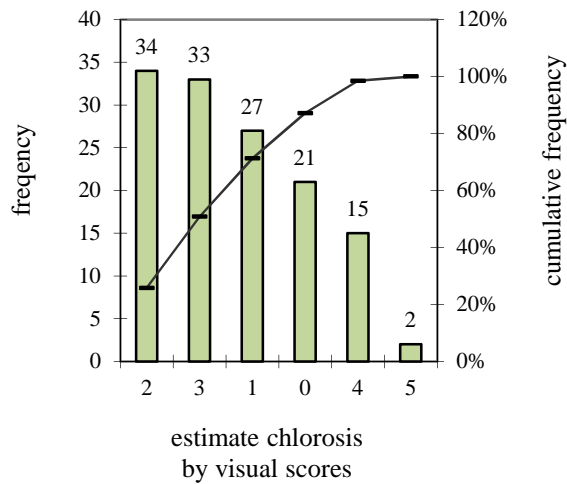
The study included field measurements of the intensity of chlorosis and laboratory analysis of soil and plant material. Field measurements of the intensity of chlorosis performed nondestructive method using chlorophyll meter (SPAD 502, Minolta Co., Japan) to a total of 60 locations. In site where chlorophyll meter measurements were performed, altitude (Alt) and geographic coordinates (Lat and Lon) were recorded. Chlorosis was visually assessed with scores of 0-5 (Table 1). From the chlorophyll meter data collection the relative chlorosis (%) were calculated (Formula 1) and mapped at the vector map.

At selected locations from which the chlorophyll meter measured the intensity of chlorosis, soil samples were taken from two depths (0-30 cm and 30-60 cm) as well as samples of plant material (leaf and petiole vines). The air-dried soil samples were determined pH values (pH in H<sub>2</sub>O and 1 mol dm<sup>-3</sup> KCl) (Vukadinovic and Berti, 1988). The content of available phosphorus and potassium (P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O) was determined using AL method. Humus content in the soil samples was determined by bichromate method (Vukadinovic and Berti, 1988), and the amount of carbonates in the soil was determined by measuring the volumetric according to Scheibler (Bogdanovic et al., 1966). Samples of leaves and stems of vines were destroyed by wet digestion (mixture of sulfuric and perkloridne acid and hydrogen peroxide), (Vukadinovic and Berti, 1988). Concentration of the iron was determined directly from the stock solution by atomic absorption spectrophotometry (AAS). All data were statistically analyzed by the usual methods.

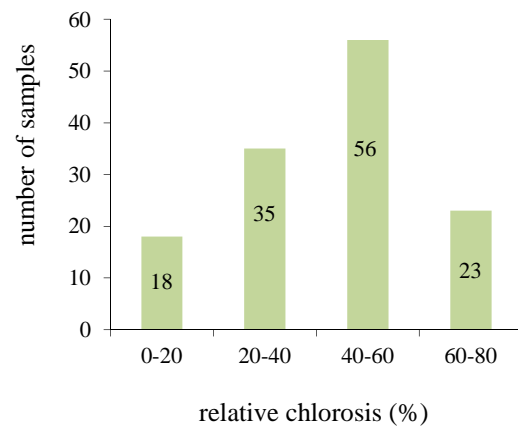
## Results and discussion

During 2007 field research on maize covered 132 sites on transect Beli Manastir - Osijek – Županja. Visual estimation of the crop chlorosis and determination of the total concentration of the chloroplast pigments by chlorophyll meter were performed on all 132 plant samples.

Frequency distribution shown that out of 132 samples only two sites (1.52%) was estimated by grade 5 (occurrence of chlorosis in more than 80% of plants), 15 samples (11.36%), with an estimate of grade 4, 33 samples (25%) with an estimate of grade 3, 34 sample (25.75%), with an estimate of chlorosis grade 2 while 21 samples (15.91%) had no symptoms of chlorosis (Graph 1). The average estimate chlorosis for maize crop was grade 2, while the CV% was very high, reaching 65.09 (Jug et al., 2008). Relative chlorosis has averaged 41.42% with a CV% of 48.84%. Maximum calculate relative chlorosis was 75.25%. Distribution of the relative chlorosis for maize is shown in Figure 1. By surveying from the west toward the east of the Croatia, the soil pH value is increasing, which is leading toward the iron and zinc deficiency and causing the appearance of the chlorosis.



Graph 1. Frequency distribution estimates chlorosis



Graph 2. Intensity of relative chlorosis



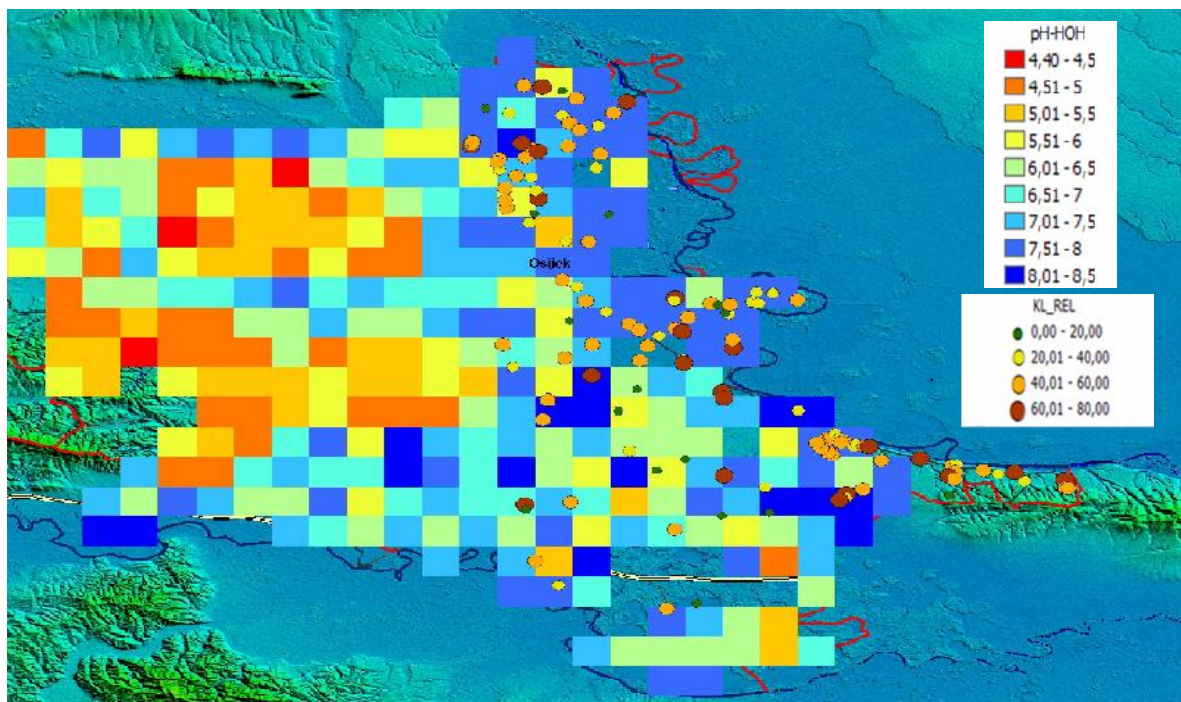


Figure 1 Map of the distribution of relative chlorosis in the east of the line of Beli Manastir - Osijek – Županija and rasterization pH values (4 x 5.5 km)

The highest relative chlorosis (60-80%) was calculated at 17.42% corn crop and the lowest (0-20%) to 13.64% of all samples. Relative chlorosis, which ranged between 40 and 60% was found in 42.42% of the samples, and in 26.52% of the samples relative chlorosis ranged 20-40% (Graph 2).

Maize is very sensitive to zinc deficiency (Mengel and Kirkby, 2001). Zn deficiency was recorded in almost all localities where chlorosis was spotted (Figure 1). In addition to the symptoms of zinc deficiency iron deficiency was also observed (especially in the area of Baranjsko brdo, Figure 2), and less frequent the deficiency of magnesium and manganese. In calcareous soils, the presence of  $\text{CaCO}_3$ , directly or indirectly, in addition to the availability of iron affects the availability of nitrogen, phosphorus, magnesium, potassium, manganese, zinc and copper (Marshner, 1995; Obreza et al., 1993). Acute zinc deficiency on the study area was expected, because the increase in soil pH reaction reduces the bioavailability of zinc in calcareous soils in poorly accessible form.

The aim of vegetation pots trial was investigate the causes of chlorosis in greenhouse conditions on maize depending on soil moisture. The soil used for vegetation trials in pots was calcareous (Table 2). Type of soil was regosols with (A)-C type of soil profile.

Table 2 Chemical properties of the soil and the concentration of trace elements

layer	pH		OM %	mg (100 g) <sup>-1</sup>		CaCO <sub>3</sub> %	IDG %	mg kg <sup>-1</sup>			
	H <sub>2</sub> O	KCl		P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O			Fe	Cu	Mn	Zn
(A)	8.61	8.01	1.14	3.1	9.72	19.85	7.0	9.11	5.31	12.17	1.52
C	8.76	8.11	0.56	0.7	6.40	17.96	5.5	4.39	0.96	2.28	0.42

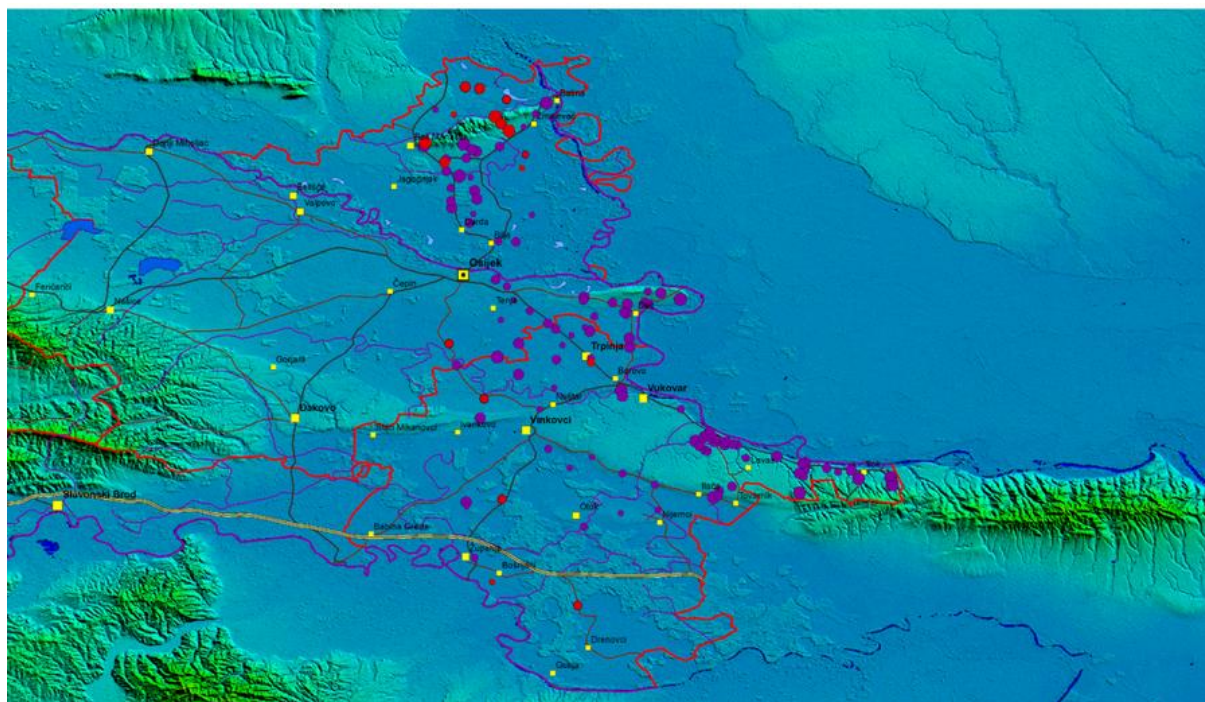


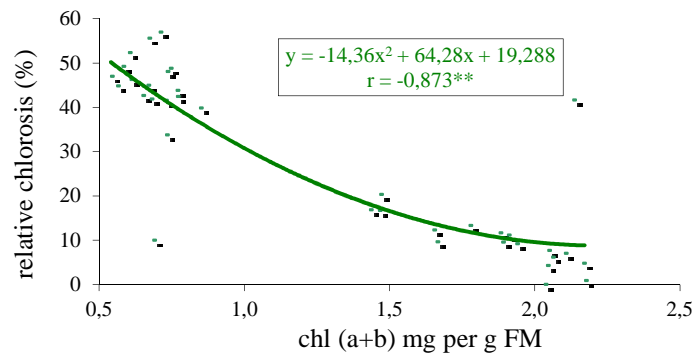
Figure 2 Fe and Zn deficiency on maize

Higher relative chlorosis (43.88%) was recorded in maize on 100% FWC treatments (Table 3) as result of reduced synthesis of chloroplast pigments. Similar results were presented by Tong Yue et al. (1987)

Table 3 Value for relative chlorosis (%), chlorophyll (a+b) (mg g<sup>-1</sup> fresh matter) and concentration of iron (µg g<sup>-1</sup>) according two different soil water levels

FWC	Kl_rel (%)	chl (a+b)	Fe
50%	11.29 <sup>b</sup>	1.83 <sup>a</sup>	72.67
100%	43.88 <sup>a</sup>	0.73 <sup>b</sup>	72.58
$\bar{x}$	27.58	1.28	72.63

Highly significant negative correlation was found between the relative chlorosis and total concentration of chloroplast pigments (Graph 3). Higher relative chlorosis indicates lower SPAD values (chlorophyll meter readings) which are in accordance with research Bavaresco et al. (2005) and Yadava (1986). Same authors reported positive correlation between SPAD readings and content of chlorophyll.



Graph 3 Correlation between relative chlorosis and concentration of chlorophyll (a+b)

Barwinsky and Remphrey (2008) revealed the presence of highly significant linear correlation between SPAD readings and concentration of chloroplast pigments in the Chinese maple leaves. Plants suffering from lime chlorosis often have a lower concentration of iron in relation to normal plants, although the Fe concentration may be equal to or even greater than in plants without visible symptoms of chlorosis (Marschner, 1995; Pestana et al., 2003). In this study, the difference in the concentration of iron in the shoots of maize in dry and wet conditions was not statistically justified, although visually maize was grown in the wetter conditions had more pronounced symptoms of iron deficiency.

These results suggest the possibility that the concentration of iron may be the same or even higher in chlorotic leaves as a possible consequence of physiological inactivation of iron (Mengel et al., 1984 a, b). Mengel (1994) reported the frequent high concentration of iron in chlorotic leaves compared to normal green leaves, and that this phenomenon indicates the possibility that the occurrence of chlorosis on calcareous soils is not always associated with the adoption of iron by the roots and its translocation to other parts of the plant, but it often depends on the utilization of iron in the leaves. This has been termed the “Fe chlorosis paradox” (Morales et al. 1998; Römheld 2000), and suggests that part of the Fe acquired from the soil by Fe-deficient plants could be immobilized and accumulated in inactive forms somewhere in the leaf (Morales et al. 1998).

Iron chlorosis is a more complex phenomenon in fruit trees than in annual crops (Tagliavini et

al., 2000a). Symptoms of iron chlorosis often start as soon as buds open, likely as a result of insufficient storage of Fe, or develop throughout the vegetative season as a consequence of plant demand being excessive in respect to Fe availability (Tagliavini and Rombolà, 2001). In general, chlorosis occurs more frequently in spring when rainfalls cause a raise in soil bicarbonate concentration in a period of intense Fe demand. If soil conditions after that period improve, new leaves appear green, but those previously chlorotic unlikely re-green. Fruit yield losses caused by leaf chlorosis also depend on the degree and the period the chlorosis develop and, in general, critical periods coincide with blooming and fruit set. Research on grape vine was conducted during the growing season 2009 in 60 locations in Baranja and Erdut vineyards whose average age was 16 years (Jug et al., 2012). Chlorosis appears on higher and slope terrain on loess because of erosion and not adequate applied soil tillage systems. In soils where shallow layers are less rich in  $\text{CaCO}_3$  than deeper layers, it is likely that grape vines develops chlorosis only when they age and roots explore layers with poor conditions for Fe uptake. Soils which had been subjected to mouldboard ploughing before the plantation may present layers of fine texture, just below the ploughing depth, which could be rich in  $\text{CaCO}_3$  because of leaching from more shallow layers. Total amounts of iron in cultivated soils, would not justify the development of iron deficiency, which,

nevertheless, often occurs as a result of poor availability of iron for plants. The most expressed problem in the vineyard production is the limiting soil pH value. The highest measured pH value in the water at a depth of 30-60 cm, was 9.03, while pH in KCl was 8.28. The lowest pH values were as follows: pH (H<sub>2</sub>O) = 6.92; pH (KCl) = 6.17 (Table 4).

Table 4 Average of chemical analyzes (60 samples), coefficient of variation (CV%), minimum and maximum values

	pH KCl	pH H <sub>2</sub> O	AL-P <sub>2</sub> O <sub>5</sub> mg 100 g <sup>-1</sup>	AL-K <sub>2</sub> O mg 100 g <sup>-1</sup>	% OM	% CaCO <sub>3</sub>
average	7.62	8.45	19.60	20.17	1.55	7.70
CV%	6.28	5.52	64.11	19.44	20.74	79.92
Min	6.17	6.92	3.40	8.13	0.85	0.00
Max	8.28	9.03	63.80	31.84	2.21	21.21

At total of 34 locations very alkaline soil reaction was measured, pH>8.51; at 17 locations moderately alkaline soil reaction (8.01-8.5) were measured, while slightly alkaline reaction was determined at 5 locations. Neutral to slightly alkaline soil solution was measured at 3 sites, while only one site had a pH between 6.5-7.0. Soils with high pH value had mainly high carbonate content (Figure 3), while the largest content of CaCO<sub>3</sub> was 21.21%. On soils, which have a high pH in the presence of HCO<sub>3</sub><sup>-</sup> ions, chlorosis is a common phenomenon that occurs as iron deficiency is confirmed by research Ksour et al. (2005).

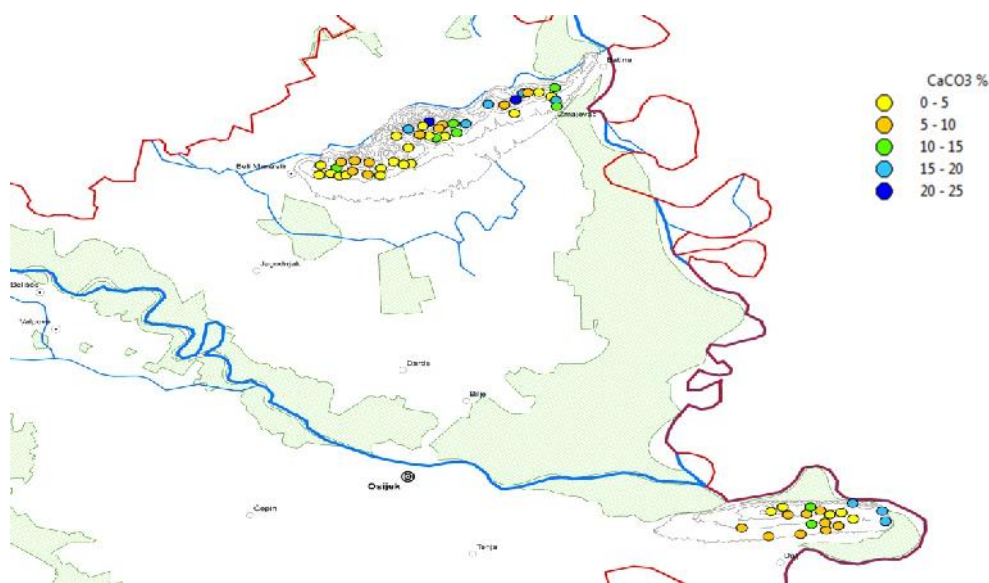


Figure 3. Distribution of CaCO<sub>3</sub> in Baranja and Erdut vineyards

The average visual chlorosis rating of grapevine was 2, while CV was very high (75.19%). Frequency distribution showed that only one site had 80-100% of grape vines being chlorotic (0.60%). At 17 sites, there was no appearance of chlorosis (28.33%), while the three sites (5.00%) to 20% of grapevine leaves appeared chlorotic. At 16 locations vines had 20-40% of leaves with symptoms of chlorosis (26.67%). At 15 sites assessment chlorosis was 3, which means that 40-60% of vines had symptoms of chlorosis (25.00%). In 8 localities chlorosis of grape vine is estimated to 4, which means that 60-80% of the vine had chlorosis symptoms. The average value of the relative chlorosis was 36.45% with a coefficient of variation of 67.36%. The highest percentage of relative chlorosis was 73.87%. Relative chlorosis is very significant, negative correlation between SPAD values.

Table 5 Average concentrations of Fe ( $\mu\text{g g}^{-1}$  DM) in the leaves (60 samples) and petioles (60 samples), the visual chlorosis rating (0-5) SPAD readings and relative chlorosis (Kl\_rel %), by calculating the value of the standard deviation (SD), coefficient of variation (CV%), minimum and maximum values of the given parameters

	leaves	petioles	visual rating	SPAD	Kl_rel
	Fe ( $\mu\text{g/g DM}$ )	Fe ( $\mu\text{g/g DM}$ )			
prosjek	161.33	109.84	1.95	23.95	36.45
Sd	41.97	27.06	1.47	9.52	24.55
KV%	26.01	24.63	75.19	39.74	67.36
Min	96.11	65.36	0.00	9.72	0.00
Max	302.31	216.58	5.00	42.08	73.87

The average iron concentration (Table 5) in grapevine leaves was  $161 \mu\text{g g}^{-1}$ , while the petioles average iron concentration was slightly lower ( $109 \mu\text{g g}^{-1}$ ). According to Reuter and Robins's (1997) it is adequate concentration of iron in the leaf and stem of the vine. According to the same authors, interveinal chlorosis presents greater help in diagnosing Fe chlorosis, compared to the obtained concentrations values of iron. In many cases, however, leaves from Fe-deficient plants grown in the field have quite high leaf Fe concentrations ( $>80\text{-}100 \mu\text{g g}^{-1}\text{DM}$ ), and there is no good correlation between leaf Fe and chlorophyll concentrations (Morales et al. 1998). In this study, the concentration of iron in the leaves and petioles of grape vines was not significantly correlated with the relative chlorosis, which can be result of physiological inactivation of iron. Gruber and Kosegarten (2002) also reported high Fe concentrations in roots and leaves on grape vines grown on calcareous soil, where plants suffered from Fe deficiency

### Conclusion

Fe chlorosis appeared on locations with high pH, high carbonate content and high altitudes. Concentration of iron in the maize leaves and grape vine leaves and petioles were relatively high, but physiologically inactive leading to inhibited synthesis of chlorophyll and finally, the appearance of chlorosis. Knowing causes that lead to the Fe chlorosis it is possible to acquire preventive action to minimize or, if possible, avoid the damage caused by Fe chlorosis.

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## WHEN ECONOMICS MATTERS IN MEETING FOOD SECURITY CHALLENGE: FOOD AFFORDABILITY AND ACCESSIBILITY IN THE MEDITERRANEAN

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### Abstract

Food security is built on four pillars: availability, access, utilization and stability. For most Mediterranean people affordability is a key factor determining access to food. This is dependent not only on food cost but also on the disposable income that can be spent on food. The paper aims at analysing food accessibility in the Mediterranean region. Secondary data from different sources (*e.g.* FAO, World Bank) were used to analyse the trends of different indicators: food affordability; food consumer price index; household food expenditure; and cereals imports dependency. According to the Global Food Security Index, food affordability is still a challenge in the Mediterranean. In March 2013, food affordability score ranged from 34.8 in Syria to 86.5 in France. The share of food consumption expenditure in total household expenditure is high; 67.3% and 43.9% in Albania and Algeria, respectively. FAO food price index increase was higher than consumer price index increase in the period 2000-2011. During the period 2005-2011, the highest increase of the food consumer price index was recorded in Egypt followed by Turkey and Algeria. FAO consumer cereal price index increased more than meat price index in the period 2004-2012. Cereals import dependency is high in all Mediterranean Arab countries; up to 80% in drought years in Algeria. Accessible and affordable diets should not be taken for granted in the Mediterranean. Adequate and consistent economic access to safe, nutritious and high quality food, even in times of crisis, is a prerequisite for achieving sustainable food and nutrition security in the Mediterranean.

**Keywords:** *food affordability, food security, Mediterranean region*

### Introduction

Recent debates on food security, nutrition and health have contributed to moving these topics higher up in the list of development programme priorities (Hassan-Wassef, 2012).

The 1996 World Food Summit definition of Food security was reaffirmed and amended officially in the 2009 Declaration of the World Summit on Food Security: “*Food security exists when all people at all times have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life.*” (CFS, 2009 in CFS, 2012). Food and Nutrition Security is a term that has been used more frequently during the past years to emphasise the need for greater integration of nutrition into food security policies and programmes (CFS, 2012). Food security is built on four pillars (CFS, 2012): (i) Food availability: sufficient quantities of food available on a consistent basis; (ii) Food access: having sufficient resources to obtain appropriate foods for a nutritious diet; (iii) Food use: appropriate use based on knowledge of basic nutrition and care; and (iv) Stability in food availability, access and utilization. Food security - including food availability, food affordability and food quality and safety - is still a challenge in many Mediterranean countries especially southern and eastern ones (The Economist, 2012).

For most people a key factor determining access to food is its affordability (Ingram, 2011). The accessibility dimension embraces Sen’s core thesis that food availability does not

guarantee that everyone is free from hunger (Sen, 1981). Affordability is dependent not only on food cost but also on the disposable income that can be spent on food (Ingram, 2011). Access to food is primarily determined by incomes, food prices and the ability of households and individuals to obtain access to social support (FAO, 2013b). For many Mediterranean consumers, income is a major barrier to freedom of nutritious and sustainable food choice. The present food economy does not deliver enough food to major parts of the population while market prices do not remunerate the costs that farmers should support to implement sustainable practices (Kickbusch, 2010).

The paper aims at analysing food affordability and financial accessibility in the Mediterranean region.

### **Material and methods**

Secondary data from different sources (*e.g.* FAO, World Bank, ILO) were used to analyse the trends of different indicators: food affordability score, food consumer price index, household food expenditure, and cereals imports dependency. The geographical coverage of this study is similar to that of the Mediterranean Strategy for Sustainable Development including 11 Northern Mediterranean and Balkan countries (Albania, Bosnia and Herzegovina, Cyprus, Spain, France, Greece, Croatia, Italy, Montenegro, Malta and Slovenia) and 10 Southern and Eastern Mediterranean countries (Algeria, Egypt, Israel, Lebanon, Libya, Morocco, Palestinian territories, Syria, Tunisia and Turkey).

The food consumer price index aims to measure the change in food and non-alcoholic beverages consumer prices over time (ILO, 1992). Data are available from LABORSTA database of the International Labour Organisation (ILO).

Food affordability score is one of the three scores used to calculate the Global Food Security Index elaborated by The Economist. Affordability category measures the ability of consumers to purchase food, their vulnerability to price shocks, and the presence of programmes and policies to support consumers when shocks occur (The Economist, 2012).

The Cereals imports dependency (CIDR) allows knowing how much of the available domestic cereal supply has been imported and how much comes from the country's own production (FAO, 2001). Data are available from FAOSTAT.

Household expenditure dedicated to food assesses the percentage of household annual income that is spent for buying food and non-alcoholic beverages (ILO, 2003). Data source is the Food security indicators (FAO, 2013a) based on elaboration of original LABORSTA data.

### **Results and discussion**

For a comprehensive and accurate assessment of food affordability and economic accessibility both micro- (household) and macro-levels (country) should be considered. At the macro-level some useful insights about food affordability are provided by cereals imports dependency ratio while food affordability score, food consumer price index and household food expenditure share allow a good appraisal of economic access to food at household level.

#### *A. Cereals imports dependency ratio*

Cereals imports dependency ratios give an idea about the level of exposure to global food price changes, which is strongly linked to food affordability and accessibility. Cereals imports dependency ratios are high especially in southern and eastern Mediterranean countries. In fact, the average cereals imports dependency ratio in the Mediterranean countries was 61% in the period 2007-09; that is much higher than the world average in the same period (15.7%). In the period 1990-92/ 2007-09 the ratio ranged between 1.6% recorded in Croatia (1990-92) and 107.7% recorded in Malta (2007-09). Cereals imports dependency ratios are particularly high in North Africa (49.9%), with respect to a developing countries average of 15.5% in the period 2007-09.



The Middle East and North Africa (MENA) region is the most food import–dependent region in the world, and net food imports are projected to rise even further in the future. With less than 5% of world population, the region accounts for more of 12% of cereal global trade (Rastoin and Cheriet, 2010). This high reliance on imported food can be attributed to both demand- and supply-side factors. Demand-side factors include rising population and changing consumption patterns due to higher income, whereas supply-side factors include limited natural resources such as land and water (Breisinger *et al.*, 2010).

What is more alarming is the fact that cereals imports dependency ratios are increasing in the majority of Mediterranean countries. The ratio increased 10.4% in the target Mediterranean countries in the period 1990-92/2007-09. The only exceptions are the Occupied Palestinian Territories, Egypt and Lebanon, where the ratios slightly decreased in the same period. Nevertheless, these results should be taken with caution as the cereals import dependency ratios remain high to very high in these three countries (35.5% in Egypt, 88.5% in Lebanon, and 96.1% in the Occupied Palestinian Territories in the triennium 2007-09). Increases were higher than 20% in Spain, Tunisia, Morocco and Portugal. Cereals, especially wheat, prices increase can have dramatic impacts on southern and eastern Mediterranean consumers as cereals per capita consumption is significant (Fig. 1).

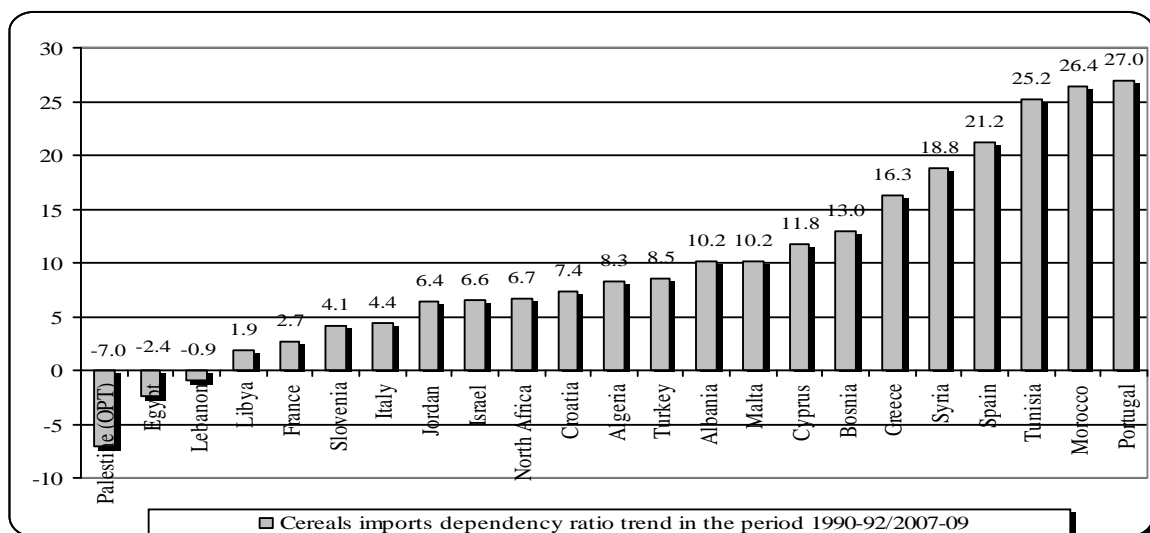


Fig. 1. Cereals imports dependency ratios trend in the period 1990-92/2007-09. Trend in Bosnia and Slovenia refers to the period 1994-96/2007-09 (Source: Authors' elaboration based on FAOSTAT data).

### B. Food consumer price index

Changes in food consumer price indices are significant among Mediterranean countries. According to LABORSTA data, taking 2000 as indices base, the increase of the consumer price indices for food was higher in North African countries (*i.e.* Algeria and Tunisia) and Turkey with respect to North Mediterranean and Balkan ones. This means that food is becoming less affordable and accessible in North Africa.

Comparing the trend of FCPI in relative terms during the period 2005-2011, it can be noticed that the highest increase was recorded in Egypt followed by Turkey and Algeria. The lowest increase was recorded in North Mediterranean countries especially Spain and Portugal, which can be due also to the effect of the financial and economic crisis on these countries (Fig. 2).

### C. Food affordability score

As of March 2013, all northern Mediterranean countries plus Israel have a very good food affordability. Food economic accessibility in the other Eastern and Southern Mediterranean countries is good to moderate. The only exception is Syria where an urgent improvement is needed in order to ensure an adequate economic access to food. That is certainly due to the civil war and unrest situation in the country.

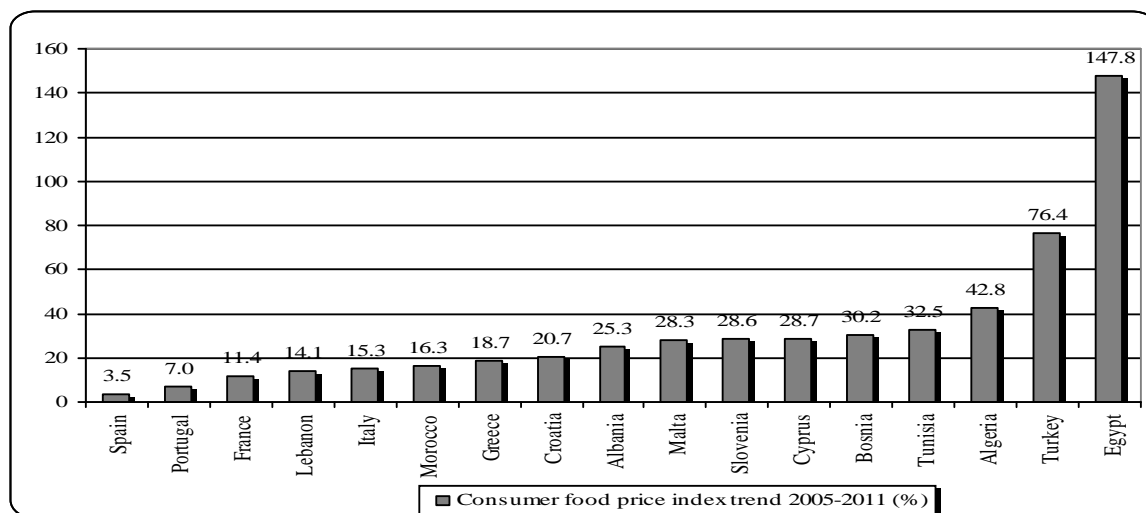


Fig. 2. Trend of consumer food price indices in selected Mediterranean countries in the period 2005-2011 (Source: Authors' elaboration based on LABORSTA data).

Considering June 2012-March 2013 period, food affordability deteriorated in most of the target Mediterranean countries especially in the PIGS countries (Portugal, Italy, Greece and Spain), that were severely affected by the global financial and economic crisis, as well as in Egypt, that was affected by the economic effects of the Arab Spring. A very low improvement was recorded in Tunisia, Israel and Algeria as well as Morocco (Table 1).

Tab. 1. Food affordability scores in selected Mediterranean countries. The score ranges between 0 and 100, where 100 = most favourable.

Country	June 2012	March 2013	Affordability change: June 2012-March 2013	Country	June 2012	March 2013	Affordability change: June 2012-March 2013
Syria	36.8	34.8	-2.0	Israel	81.2	81.4	0.2
Algeria	38.7	38.8	0.1	Greece	81.7	77.7	-4.0
Egypt	39.3	37.6	-1.7	Spain	85.7	84.0	-1.7
Morocco	50.9	51.5	0.6	Italy	85.6	84.3	-1.3
Tunisia	54.3	54.5	0.2	Portugal	82.0	80.5	-1.5
Turkey	60.1	58.6	-1.5	France	86.7	86.5	-0.2
Jordan	55.8	54.7	-1.1				

Source: After the Economic Intelligence Unit, *The Economist*.

#### D. Household food expenditure

Many poor consumers spend up to 70% of their incomes on food (Rajiv, 2010). For them, there is little latitude to offset the price rise simply by spending more (von Braun, 2008). According to Engel's law, the share of food expenditure in disposable income is expected to

decline as income levels rise. This means that poor households spend a relatively high share of their disposable incomes on food, making them vulnerable to sudden increases in food prices or losses of income (FAO, 2013b).

The highest shares of food expenditure in total consumption expenditure are recorded in Balkan and southern and eastern Mediterranean countries while the lowest ones are recorded in northern Mediterranean countries and Israel. In fact, Albanian consumers spend more than a half of the household budget for buying food products while French and Spanish consumers dedicate less than a quarter of the budget to food consumption expenditure (Fig. 3).

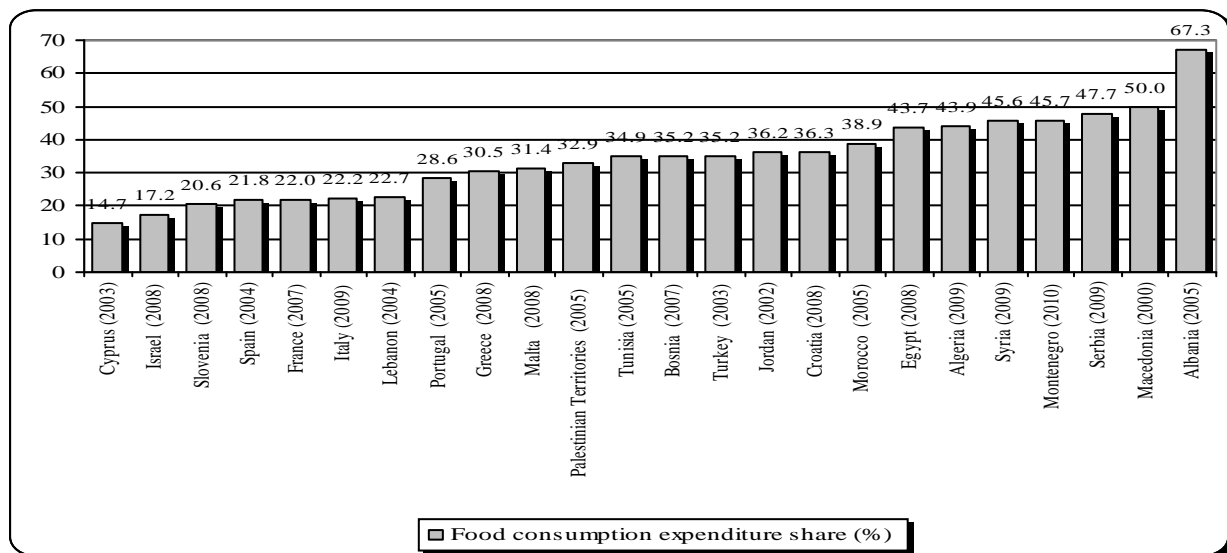


Fig. 3. Food consumption expenditure in selected Mediterranean countries (%) (Source: FAO elaboration based on ILO, LABORSTA data).

A decrease of the share of food consumption expenditure is noticeable in many Mediterranean countries: Croatia, Cyprus, Israel, Italy, Lebanon, Malta, Portugal, Slovenia, Spain, Tunisia and Turkey. The highest decrease was recorded in Balkan and southern and eastern Mediterranean countries. However, a high decrease was recorded also in Spain after joining the EU. That is probably due to the increase of income and the improvement of living conditions in the country. However, the food consumption expenditure share increased in some northern Mediterranean countries such as France and Greece.

Cereal imports and high prices are calling the socio-economic and environmental sustainability of the Mediterranean diet in question (in terms of purchasing power and food miles), particularly in certain southern and eastern Mediterranean countries. According to the World Bank (2012), higher prices particularly hurt consumers with high shares of household expenditure on food. High and volatile international food prices continue to be a big concern in the Mediterranean. Some have even cited the food price developments since 2007 as a contributing factor in the Arab Spring (Breisinger *et al.*, 2011).

### Conclusions

Food security is built on availability, access, utilization, and stability pillars. For most people a key factor determining food access is its affordability. Food affordability is still a challenge in all southern and eastern Mediterranean countries. Paradoxically, these are also the countries where there was some improvement during the last months while the situation is getting worse in most northern Mediterranean countries where consumers were badly affected by the economic crisis. Income growth and exposure to global food price changes

drive changes in affordability. Food price indices increased in the Mediterranean area in general and North Africa in particular. This puts at risk food accessibility especially for those households dedicating a large share of their consumption expenditure to food. The lowest shares of food consumption expenditure in total household consumption expenditure are recorded in northern Mediterranean while the highest ones are recorded in Balkan countries. Consumers in some Mediterranean countries still dedicate about a half of their household budget to food consumption expenditure especially in the Balkans and southern and eastern Mediterranean countries. High cereals imports dependency ratios, especially in MENA, make these countries more exposed to global food price changes and volatility. Cereals imports dependency ratios are increasing in most of the Mediterranean countries, including northern ones. Sustainable diets should be economically accessible and affordable but the presented data show that that should not be taken for granted in the Mediterranean. Adequate and consistent economic access to safe, nutritious and high quality food, even in times of crisis, is a prerequisite for achieving sustainable food and nutrition security in the Mediterranean region.

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**THE IMPACT OF LAND USE ON SOIL EROSION IN THE RIVER BASIN  
BOLJANSKA RIJEKA IN MONTENEGRO**

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**Abstract**

Soil erosion is acknowledged as a major environmental problem, threatening sustainable livelihoods around the world. Inappropriate land use and land management is often viewed as main cause of accelerated erosion rates. Therefore, modelling soil erosion rates under various land use and climate conditions is key to understand the impact of future land management and climate change on land degradation. For the Boljanska Rijeka River Basin (Polimlje, Montenegro), we studied soil erosion processes, using a series of data that are reflecting variations in land use over a period of four decades (1970-2013). The computer-graphic IntErO model was used to calculate soil erosion intensity, taking data of Forest Management Plans, Cadastre, Landsat images and Statistical Yearbooks into account. It was concluded that the condition of the vegetation cover and the land use influenced the development of erosion processes in the river basin. For the current state of land use, calculated maximal outflow from the river basin is  $212 \text{ m}^3 \text{ s}^{-1}$  and the net soil loss is  $8644 \text{ m}^3 \text{ year}^{-1}$ . This indicates that the river basin belongs in „Destruction Category V”, according to the classification system of Gavrilovic. The strength of the erosion process is medium, and according to the erosion type, it is mixed erosion. Change of the land use in structure for the period of four decades (1970-2013), in the studied river basin, decreased the soil erosion intensity by 3.95%.

**Key words:** *Soil erosion rates, Runoff, Land use, Modelling, Prediction, IntErO model.*

**Introduction**

Erosion is responsible for large soil losses. According to Poesen *et al.* (2003) in this part of Europe, erosion has led to the formation of extensive degraded areas called badlands, in which high rates of soil loss is observed (Mathys *et al.* 2003).

According to Spalevic (2011), Kostadinov *et al.* (2006), Lazarevic (1996) water erosion has affected  $13,135 \text{ km}^2$  or 95% of the total territory of Montenegro ( $13,812 \text{ km}^2$ ). Erosion caused by water is dominant in the terrains with high slopes due to complex physical and geographical conditions paired with reckless logging (Spalevic *et al.* 2012).

The exploitation of forests and the irrational use of land caused a change in land use structure (Nyssen *et al.*, 2012), and the quality of vegetation cover in the studied river. The soil and geological substrate are exposed to the impact of various agents, particularly water, temperature and gravity. A field survey shows that in some places, some ridges, gullies and ravines have appeared; at some highlands sandbanks are present.

All these facts obtained in the process of the field survey led the authors to analyse the impact of land use on soil erosion intensity in this area using a computer-graphic method.

## Material and methods

The study was conducted in the area of the river basin of Boljanska Rijeka, a right-hand tributary of the river Lim, which lies on the slopes of Kurilo (1314m) on the North, Krstac (910m) and Macino brdo (1076) from the South (Figure 1). The river basin of Boljanska Rijeka encompasses an area of 27.5 km<sup>2</sup>. It is part of the natural entity of the Polimlje region (North-East of Montenegro). The natural length of the main watercourse,  $L_v$ , is 6.5 km. The shortest distance between the fountainhead and the mouth,  $L_m$ , is 6 km. The total length of the main watercourse, with tributaries of I and II class,  $L$ , is 8.66 km.

Fieldwork was undertaken to collect detailed information on the intensity and the forms of soil erosion, the status of plant cover, the type of land use, and the measures in place to reduce or alleviate the erosion processes. Morphometric methods were used to determine the slope, the specific lengths, the exposition and form of the slopes, the depth of the erosion base and the density of erosion rills.

We drew on the earlier pedological work of Fustic and Djuretic (2000), who analysed the physical and chemical properties of all the Montenegrin soils, including those in the study area of Boljanska Rijeka. Furthermore, some pedological profiles had been reopened, and soil samples were taken for physical and chemical analysis. The granulometric composition of the soil was determined by the pipette method; the soil samples were air-dried at 105°C and dispersed using sodium pyrophosphate. The soil reaction (pH in H<sub>2</sub>O and nKCl) was determined with a potentiometer.





Polimlje: 43.245703 N, 19.580383 E (North); 42.508046 N, 19.905853 E (South);  
43.148092 N, 19.485626 E (West); 42.963960 N, 20.120087 E (East).

Figure 1: Study area

The total carbonates were determined by the volumetric Scheibler method; the content of the total organic matter was determined by the Kotzman method; easily accessible phosphorous and potassium were determined by the Al-method, and the adsorptive complex (y1, S, T, V) was determined by the Kappen method.

Reduction of soil erosion to preserve soil quality and to maintain land productivity constitutes a major challenge for mountainous soils. Soil erosion can be reduced by appropriate land management. It requires both the collection of field data and the predictive model for the evaluation of different management scenarios for the protection of soils. Field measurements of erosion and sedimentation using classical techniques is time-consuming and expensive (Bujan *et al.* 2000). The modelling of the erosion process has progressed rapidly, and a variety of models have been developed to predict both the runoff and soil loss (Zhang *et al.* 1996).

Most of the methodologies remained at the qualitative (descriptive) level, relying on empirical evidence and expert subjective evaluation of the conditions. In the South-Eastern European Region two methodologies have achieved the required level of standardization of research procedures to minimize subjective errors of the researchers, which allows obtaining uniform results, tracking the state of changes in erosion intensity over a period of time.

The first method is the "*Soil Loss Equation*" of the U.S. Soil Conservation Services, further improved, now known as USLE (*Universal Soil Loss Equation*). Another method is the "*Erosion Potential Method - EPM*", created, developed, and calibrated in Yugoslavia (Gavrilovic, 1972).

Both of these methods are standard for use in agriculture and water management, according to its primary purpose, but it should be noted that the accuracy of the USLE method ends for the surfaces with the slope of less than  $7^{\circ}$  as it is developed for determining of erosion processes for agricultural production. "*Erosion Potential Method*" covers a wide range of soil erosion intensities.

According to previous experience, and verifications (Spalevic, 2011, 2012, 2013) the most reliable method for determining the sediment yields and the intensity of the erosion processes for the area of North of Montenegro is the *Erosion Potential Method*.

Blinkov and Kostadinov (2010) evaluated applicability of various erosion risk assessment methods for engineering purposes. Factors taken into consideration depended on scale,



various erosion tasks as well as various sector needs. The EPM was the most suitable on catchment level for the watershed management needs in this Region.

The use of computer-graphics in research on runoff and the intensity of soil erosion have been demonstrated in Montenegro, specifically in the Region of Polimlje (Spalevic *et al.* 2013, 2012, 2011, 2007, 2004, 2003, 2001, 2000, 2000a, 1999, 1999a), Fustic and Spalevic (2000). That approach was used in the research on the river basin of Boljanska Rijeka. We used the **I**ntensity of **E**rosion and **O**utflow (IntErO) program package (Spalevic, 2011) to obtain data on forecasts of maximum runoff from the basin and soil erosion intensity. EPM is embedded in the algorithm of this computer-graphic method.

## Results and discussion

### *Physical-geographical characteristics and erosion factors*

The river basin of Boljanska Rijeka stretches from its inflow to Lim ( $H_{\min}$ , is 550 m) to the tops of the Kofiljaca, where the  $H_{\max}$  is 1314 m. There is a flat area in and around the village of Rasova, mild slopes around the village Boljana and steep slopes in the upper part of the river basin on the slopes of Kofiljaca. The average river basin decline,  $I_{sr}$ , is 41.6% and indicates that in the river basin prevail very steep slopes. The average river basin altitude,  $H_{sr}$ , is 736.33 m; the average elevation difference of the river basin,  $D$ , is 186.33 m.



Figure 2: Details from the River basin: Zminjica (left), Boljanska River (right)

### *Climatic characteristics*

The area is characterised by dry summers; rainy autumns and springs; and cold winters. The absolute maximum air temperature is  $39.2^{\circ}\text{C}$ . Winters are severe, so much so that negative temperatures can fall to a minimum of  $-27.6^{\circ}\text{C}$ . In terms of rainfall, there are two characteristically rainy periods of the year: the first-cold period (October-March) and the second-warm period (April-September).

The amount of torrential rain,  $h_b$ , is 84.7 mm. The average annual air temperature,  $t_0$ , is  $8.9^{\circ}\text{C}$ . The average annual precipitation,  $H_{\text{year}}$ , is 873 mm.

### *The geological structure of the area*

In the structural-tectonic sense, the studied area belongs to the Durmitor geotectonic unit of the inner Dinarides of Northern and North-eastern Montenegro (Zivaljevic, 1989).

The **geological** structure of that part of Montenegro consists mainly of Paleozoic clastic, carbonate and silicate volcanic rocks and sediments of the Triassic, Jurassic, Cretaceous-Paleogene and Neogene sediments and Quaternary.

The coefficient of the region's permeability,  $S_1$ , is calculated on 0.96. The structure, according to the permeable products from rocks is presented in the Figure 3.

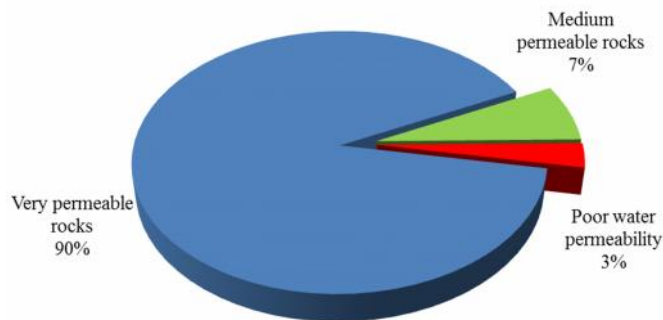


Figure 3: Structure of the Boljanska River basin according to the permeable products from rocks

#### *Soil characteristics of the area*

Going from the inflow of the Boljanska Rijeka past Lim to the surrounding mountainous terrain, the most common soil types are Brown district (acid) soils (84%) and Brown eutric soils (15%); close to the inflow of Boljanska River to Lim, Alluvial-deluvial soils.

#### *Vegetation and Land use*

The studied area is located in Dinaridi Province of the Middle-Southern-East European mountainous biogeographical region. The dominant type of vegetation in the studied river basin is forests accounting for two thirds of the total vegetation cover.

Plant communities of the studied area belongs to the following classes of vegetation: *Quercus-fagetum* Br.-Bl. et Vlieger 37.; *Quercetum robori-petraeae* br.-Bl. et Tx. 43.; *Alnetum glutinosae* Br.-Bl. et Tx. 43.; *Arhenantheretum* Br.-Bl. 47.; *Festuco brometum* Br.-Bl. et Tx. 43.; *Plantaginietum majoris* Tx. et Prsg. 50; *Salicetum herbaceum* Br.-Bl. 47.

On the vertical profile, River basin of Boljanska river is differentiated from the following forest communities: (1) *Quercetum petraeae-cerridis*, Lak. Mostly in the southern exposure of the valleys of the main watercourse, and the lower parts of its tributaries; (2) *Quercetum petraeae montenegrinum*, Lak. On the hilly parts of the river basin; (3) *Fagetum montanum* differentiated into several associations (Curovic *et al.* 2011) and (4) *Abieti - Fagetum moesiaca* Bleck and Lak.

Most of the river basin is covered with low beech forests (*Fagetum montanum*) and forests of Sessile oak and Turkish oak (*Quercetum petraeae-cerridis*). On the southern exposures and lower altitudes there are forests of Sessile oak and Turkish oak (*Quercetum petraeae-cerridis*). A narrow belt near the river in the lower part of the river basin is covered with hygrophilic forest (*Alnetum glutinosae*, *Salicetum herbaceum*). At the higher parts of the basin there are mixed of broadleaves and deciduous tree species (*Abieti - Fagetum moesiaca*). In last decades climate change on forest ecosystems affected moving of the vegetation vertical layout belts (Curovic and Spalevic, 2010).

The coefficient of the vegetation cover,  $S_2$ , is calculated on 0.7. The coefficient of the river basin planning,  $X_a$ , is 0.5. Of the total river basin area, related to the river basin structure, degraded forests are the most widespread form (40.67%). Further proportion is as follows: well-constituted forests (21.9%), arable land - plough-lands (14.07%), meadows (12.35%), grassland (6.76%), and orchards (4.25%). The structure of the river basin of Boljanska Rijeka, according to the land use is presented in Figure 4.

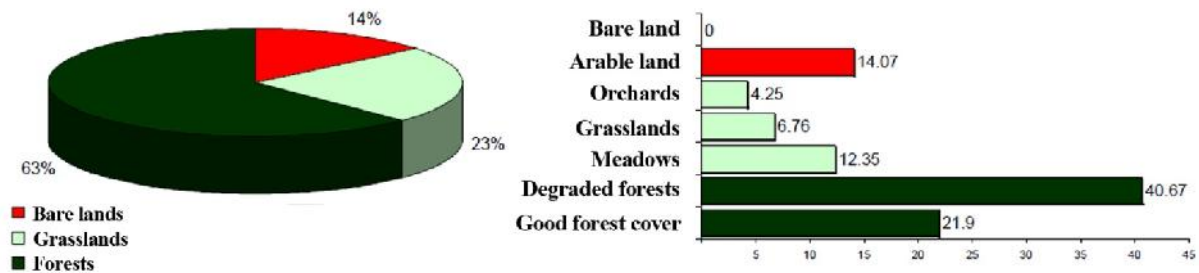


Figure 4: Land use in the river basin of Boljanska Rijeka (year 2013)

### *Characteristics of the basin regarding issues of soil erosion and runoff*

The dominant erosion form in this area is surface runoff, but more severe forms of erosion, such as rills, gullies and ravines, occur also.

The erosion causes some places to lose fertile land, and results in sterile alluvial deposits on the fertile soils of the small alluvial terraces close to the main watercourse. Surface erosion has taken place in all the soils on the slopes, with the effect that this erosion is most pronounced on the steep slopes with scarce or denuded vegetation cover.



Figure 7: Detail from the basin - Kurilo

We used the software IntErO for calculation of the soil erosion intensity and the maximum outflow.

Coefficient of the river basin form,  $A$ , is calculated on 0.95. Coefficient of the watershed development,  $m$ , is 0.34 and average river basin width,  $B$ , is 2.12 km. ( $A$ )symmetry of the river basin,  $a$ , is calculated on 0.69 and indicates that there is a possibility for large flood waves to appear in the river basin.

Density of the river basin network,  $G$ , is calculated on 0.32 and indicates there is low density of the hydrographic network. The height of the local erosion base of the river basin,  $H_{leb}$ , is 764 m. Coefficient of the erosion energy of the river basin's relief,  $E_r$ , is 106.28.

Coefficient of the river basin erosion,  $Z$ , is 0.537. The strength of the erosion process is medium, and according to the erosion type, it is mixed erosion.

Production of erosion material in the river basin,  $W_{god}$ , is calculated on  $29484 \text{ m}^3 \text{ year}^{-1}$ ; Coefficient of the deposit retention,  $R_u$ , on 0.293. For the current state of land use, calculated maximal outflow from the river basin is  $212 \text{ m}^3 \text{ s}^{-1}$ . Real soil losses,  $G$  year, are calculated on  $8644 \text{ m}^3 \text{ year}^{-1}$ ; and the specific real soil losses on  $315 \text{ m}^3 \text{ km}^{-2} \text{ year}^{-1}$ .

### *The impact of Land use on Soil erosion*

Using a series of data of Forest Management Plans, Cadastre, Landsat images and Statistical Yearbooks that are characterizing variations in land use over the period of four decades (1970-2013) it is concluded that:

- Area under forests is prevailing covering around two thirds of the area; meadows, pastures and orchards covers around quarter of the studied river basin; ploughed land and ground without grass vegetation are decreasing from 16% (1970) to 14% (2013). The land use changes are presented on Figure 8.

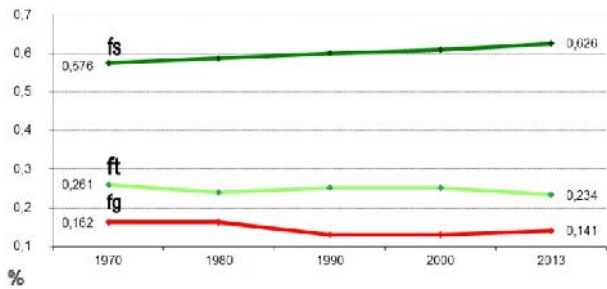


Figure 8: Land use (period 1970-2013)

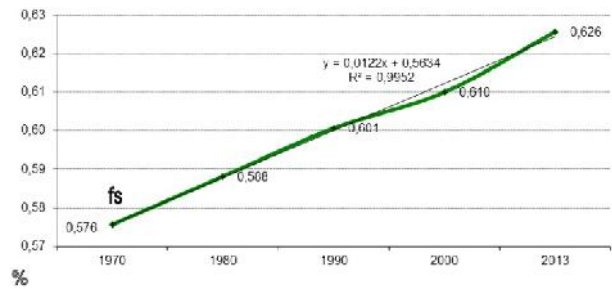


Figure 9: Forests (fs)

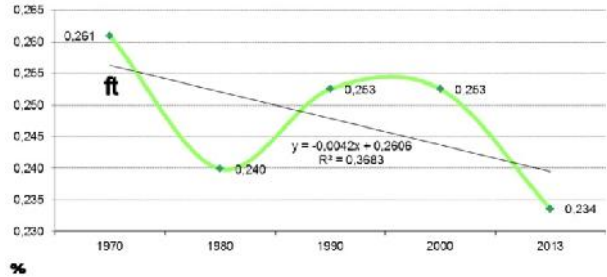


Figure 10: Orchards and meadows (ft)

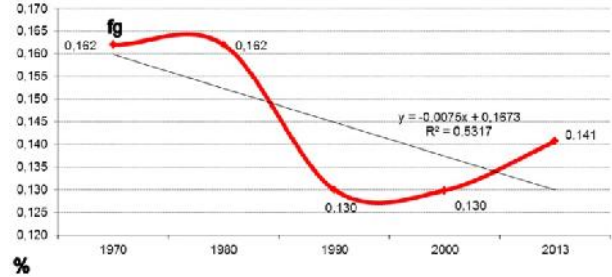


Figure 11: Bare lands, & Plough-lands (fg)

- Increase of areas under the forests over the period of time is presented on Figure 9.
- Decrease of the areas under the grassland is presented on Figure 10.
- The areas under bare lands & plough-lands on figure 11.

Figure 12. Stanica Scekic showing the change of land use: from wheat (1980) to meadows (2010).



The impact of land use on soil erosion intensity over the period of four decades in the Boljanska river basin is presented in the Figures 13 – 16.

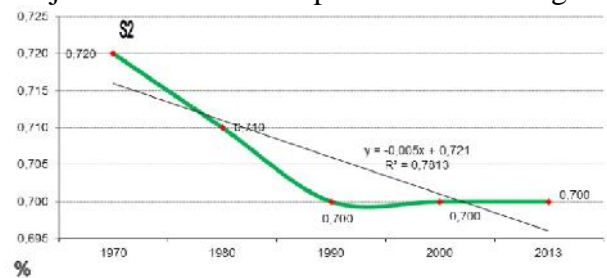


Figure 13: Vegetation cover coefficient

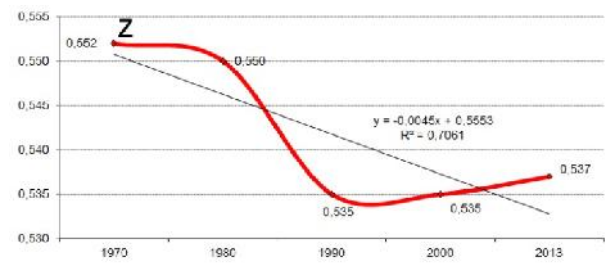


Figure 14: River basin erosion coefficient

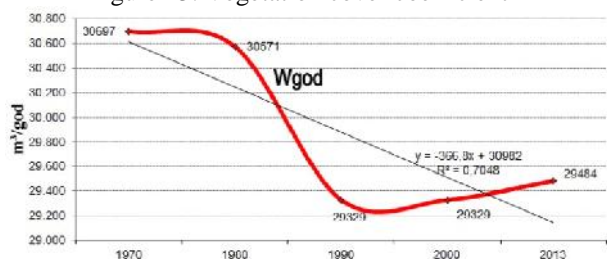


Figure 15: Production of erosion material in the basin

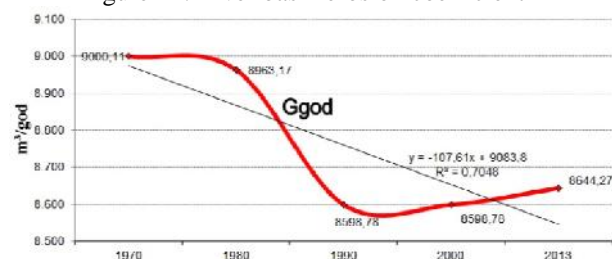


Figure 16: Real soil losses

Those graphics explained that the value of the Vegetation cover coefficient (S2) stabilised on 0.7 after the decade 1990. The value of the River basin erosion coefficient (Figure 14) is

decreasing from 0.552 (1970) to 0.537 (2013). Production of erosion material (Figure 15) over the period of four decades in the river basin is also decreasing from 30697 m<sup>3</sup> year<sup>-1</sup> (1970) to 29484 m<sup>3</sup> year<sup>-1</sup> (2013). Real soil losses for the river basin per year (Figure 16) are decreased for 356 m<sup>3</sup> year<sup>-1</sup>, from 9000 m<sup>3</sup> year<sup>-1</sup> (1970) to 8644 m<sup>3</sup> year<sup>-1</sup> (2013).

### Conclusion

Many factors have influenced the erosion processes in the territory of the river basin of Boljanska Rijeka. The most significant factors are the area's climate, relief, geological substrate and pedological composition, as well as the condition of the vegetation cover and the land use.

Maximal outflow (appearance of 100 years) from the river basin,  $Q_{\max}$ , is 212 m<sup>3</sup> s<sup>-1</sup> and is suggesting the possibility of a large flood. The strength of the erosion process is medium, and the erosion type is mixed erosion. The calculated soil losses were 8644 m<sup>3</sup>/year (315 m<sup>3</sup>/km<sup>2</sup>/year).

**Change of the land use in structure for the period of four decades (1970-2013), in the studied river basin, according to our analyses, decreased the soil erosion intensity for 3.95%.**

### Acknowledgement

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## **1. PLANT PRODUCTION**



## RESPONSE OF DIFFERENT MAIZE HYBRIDS TO LIMING

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### Abstract

Soil acidity is the major growth-limiting factor for more than 40% of the world's arable land. It is assumed that over 50% of soils in Serbia belong to a group of acid soils and that this percentage is permanently increasing. Species and genotype within species greatly differ in their tolerance to acid reaction and Al toxicity. As the majority of cultivated plants require slightly acid, neutral to alkaline reaction of the soil, a very small number of crops can tolerate an extremely acid reaction and conditions of these types of soils that have been gradually expanding. Six maize hybrids were grown under field conditions on acid soil (pH in 1N KCl = 4.41) in three replicates at the stationary field experiment on the arable land of Kraljevo (pseudogley) for 2-year period (the growing seasons 2007–2008). Fertilization treatments were the following: a = unfertilized (control); b = lime – 3 t ha<sup>-1</sup>; c = lime – 5 t ha<sup>-1</sup>. In addition to the statistical differences between various hybrids greatest difference in yield was achieved when comparing NS 5010 and NS 6010 approximately 1762 kg ha<sup>-1</sup> dry grain. Two-year results, which are used in this study showed that the maize yield was higher in 2008 year which had a favorable agro-meteorological characteristics. Individual highest yield was obtained with hybrid NS 6010 in 2008 year and amounted to 11930 kg ha<sup>-1</sup>. Genetic adaptation of plants to acid reaction and Al toxicity may provide a sustainable strategy to increase crop yield in the tropics at relatively low costs and low environmental impacts.

**Key words:** soil acidity, liming, maize hybrids.

### Introduction

Acid soils cover approximately 3950 million hectares, corresponding to about 30% of the total ice-free land area on the Earth. Acid soils are found throughout the world, with 41% in the Americas, 26% in Asia, 17% in Africa, 10% in Europe, and 6% in Australia and New Zealand (Von Uexkull and Mutert, 1995). Low pH and Al<sup>3+</sup> stresses are the major causes of poor plants growth in acidic soils (Bose et al., 2010). High concentration of toxic aluminium are the most important cause of reduced yields associated with soil acidity (Tang et al., 2003). Acid soils have in some cases toxic levels of Al and Mn, deficiency of Ca, Mg, P, K and Mo as well. These characteristics limit the fertility of acid soils and inhibit root development, leading to low water and nutrient uptake and low maize yields (Welcker et al., 2005).

Maize grain-yield increase has been obtained on acid soils through selection for tolerant cultivars in tropical maize populations (Pandey and Gardner, 1992). Most breeding work designed at increasing productivity on acid soil, focused on tolerance to Al toxicity (Garvin and Carver, 2003). Thus, the use of acid-soil tolerant cultivars might thus have the potential of bringing unproductive acid soils into productive cultivation in the short term with limited

inputs especially lime (Clark, 1997). Lime is widely known as the most effective mean for correcting soil acidity. The direct effect of soil amendment through lime is a change in soil pH. Its application usually results in significant reduction of exchangeable Al (Lukin and Eppin, 2003) allowing for a more efficient uptake of N and P (Raij and Quaggio, 1997). Osei (1995) studied the effects of different lime application rates and time of application on some chemical properties of an acid soil. Significant increase in pH (> 28%) was obtained at all soil sampling depths. Available P also increased significantly (> 90%). Exchangeable Al was completely eliminated when most of the soil samples had pH > 5.0. The results clearly indicated that liming as a management practice, could be used to alleviate or prevent acidification of pseudogley. Acid soils (mainly pseudogley) covering considerable part of Serbia territory, especially in a ak-Kraljevo basin. It is estimated about 32.000 ha of pseudogley in this part of Serbia (Dugali, 1998). Besides liming and adequate fertilization practice, using acid soil-tolerant maize cultivars provides an environmentally friendly, inexpensive, and permanent solution, contributing to sustainable crop production on acid soils.

The aim of this study was to test the response of maize hybrids to liming.

## Material and Methods

### The field experiment

Six maize (*Zea mays* L.) hybrids (NS 300, NS 4015, NS 444, NS 5043, NS 5010, NS 6010) were grown at stationary field experiment on the arable land of Kraljevo (pseudogley) for 2-year period (the growing seasons 2007–2008) on fertilization treatments as follows (kg ha<sup>-1</sup>): a = unfertilized (control); b = lime – 3 t ha<sup>-1</sup>; c = lime – 5 t ha<sup>-1</sup>; The experiment were conducted in three replicates. Waste of the Serbian Glas Factory in Para in commercially named „Njival Ca“ (calcite 98.5% CaCO<sub>3</sub>; granulation < 0.1 mm) was used as liming material. It was incorporated in soil in autumn 2006 before ploughing. The maize hybrids were developed in the Novi Sad Institute of Field and Vegetable Crops. Maize was sown manually in term end April/beginning May on planned density 57142 seeds ha<sup>-1</sup> (distance in row 25 cm; interrow spacing 70 cm). Maize harvest was also made manually by harvesting two internal rows. Maize grain yields were calculated on 14% moisture basis and 90% realization of theoretical plant density. Soil sampling was made by the auger to 30 cm of depth. Soil reaction were determined according to ISO (1994). Extraction with the ammonium-lactate (AL-method) was used for determination of plant available P and K. Statistical analyses were performed according Mead et al. (1996).

### Soil characteristics

The experimental plot has acid reaction with poorly levels of plant available phosphorus and base saturation less than 50% (Table 1).

Table 1. Soil characteristics (0–30 cm depth)

pH		Humus (%)	Total N (%)	AL-method		Adsorption complex characteristics			
H <sub>2</sub> O	KCl			P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	T-S	S	T	V
				(mg 100 g <sup>-1</sup> )			(mmol <sup>+</sup> /100 g)	(me/100 g)	(%)
5.44	4.41	2.59	0.168	5.40	14.10	11.41	11.15	22.56	49.24

### Weather characteristics

Weather conditions had considerable impacts on field crop yields, including maize. Yield variations among years for the short period is mainly result of weather conditions, especially precipitation and temperature regime during growing season.

From the data in tab. 2, one can see that the average air temperature over 2007 and 2008 was 19.3°C and 18.9°C, i.e. by 1.6°C and 1.2°C higher than the long-term average. The total precipitation amount was 348 mm in 2007 and 383 mm in 2008, significant less than the long-term average, respectively.

Table 2. Meteorological data (Kraljevo Weather Bureau) for 2007–2008

The period	Precipitation (mm) and mean air-temperatures (°C) in Kraljevo							Total	Mean
		April	May	June	July	August	Sept.		
2007	mm	17	126	31	19	63	92	348	19.3
	°C	12.8	18.1	22.1	24.8	23.1	15.1		
2008	mm	22	47	116	42	61	95	383	18.9
	°C	13.2	18.5	21.8	21.6	22.4	15.8		
1961–1990	mm	58	89	101	78	64	53	443	17.7
	°C	11.3	16.4	19.6	20.9	20.7	17.0		

In general, low yields of maize is in connection with drought and the higher air-temperatures, especially in July and August (Čalović et al., 2011).

### Results and Discussion

Liming increases the uptake of nutrients, stimulate biological activity and reduce toxicity of heavy metals. Liming raises the soil pH and causes the aluminum and manganese to go from the soil solution back into solid (non-toxic) chemical forms (Krstić et al., 2012).

Lime application generally and significantly increased the maize grain yield. The grain yields of maize was influenced by liming in all two years and yield was significantly increasing at all liming treatments compared with control (tab. 3).

Table 3. The effects of various hybrids and liming on maize yield

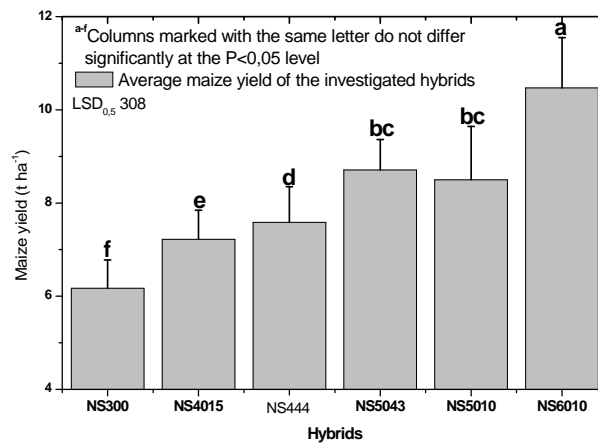
Hybrids	Liming						Average/ Hybrids
	0 t ha <sup>-1</sup>		3 t ha <sup>-1</sup>		5 t ha <sup>-1</sup>		
	2007	2008	2007	2008	2007	2008	
NS 300	5.256	5.670	6.186	6.470	6.346	7.096	<b>6.171 f</b>
NS 4015	6.033	6.993	7.243	7.516	7.590	7.940	<b>7.219 e</b>
NS 444	6.316	7.180	7.433	8.226	7.733	8.623	<b>7.585 d</b>
NS 5043	7.670	8.183	9.143	9.336	8.550	9.373	<b>8.709 bc</b>
NS 5010	6.833	7.696	8.146	9.440	8.626	10.236	<b>8.496 bc</b>
NS 6010	8.726	10.563	9.836	11.450	10.320	11.930	<b>10.471 a</b>
<i>Average/Liming</i>	<b>7260 C</b>		<b>8369 B</b>		<b>8697 A</b>		

<sup>A-C</sup>Values in rows of each doses followed by similar letters do not differ significantly at *P* 0.05

<sup>a-e</sup>Values in column of average hybrid yields followed by similar letters do not differ significantly at *P* 0.05

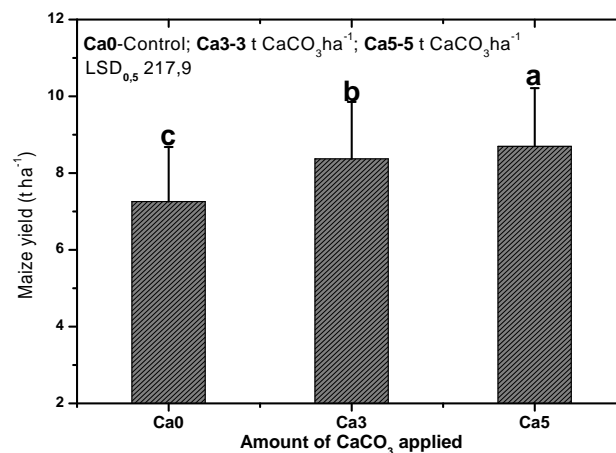
The statistical differences between various hybrids were observed, however difference in yield of NS 5043 and NS 5010 was not distinguished. In addition to that yield variability increased as growing period extended. Two-year results, which are used in this study showed that the maize yield was higher in 2008 year which had a favorable agro-meteorological

characteristics. Individual highest yield was obtained with hybrid NS 6010 in 2008 year and amounted to 11930 kg ha<sup>-1</sup> (tab. 3; graf. 1).



Graf. 1. The average yield of maize hybrids after of different doses of lime

In addition, obtained data showed that the hybrid NS 6010 in the control variant (no lime added) has a higher yield compared with the lower hybrid maturity group. Addition of 3 t ha<sup>-1</sup> CaCO<sub>3</sub> resulted with 15% increase in yield, whereas with 5 t ha<sup>-1</sup> of lime 20% higher maize yield was observed across all hybrids (graf. 2).



Graf. 2. Average maize yield after lime application

Similar results of applying liming materials were attained by Trávník et al. (1998); Ernani et al. (2002) and Kova evi et al. (2006). For example, Kova evi et al. (2006) applied carbocalk (waste of sugar factory, 39% CaO) in five rates up to 90 t ha<sup>-1</sup>. Liming with carbocalk resulted with considerable increases of field crop yields (maize–maize–sunflower–barley rotation) as follows: up to 50% and 36% (maize for 2001 and 2002, respectively), up to 49% (sunflower 2003) and up to 30% (barley 2004). In general, by application of carbocalk in level of 90 t ha<sup>-1</sup>, yields considerably decreased mainly to level of control as affected by overliming.

Genotypic differences for tolerance to soil acidity/Al toxicity among hybrids of maize have been reported Pandey and Gardner (1992). Lime application generally increased the grain

yield of the susceptible genotype 208% compared to 82% of the tolerant genotype and usually was associated with significant reduction in exchangeable Al (The et al., 2012).

In recent period, a new principle in solving the increase of acid soil productivity has occurred—breeding and selection of varieties, i.e. the development of hybrids of cultivated crops tolerant to adverse conditions in acid soils, first of all to increased concentration of Al ions in the nutrient solution (Welcker et al., 2005; Jovanovi et al., 2007). Chemical amendment (melioration) of acid soils (soil adaptation to the plant) and breeding and selection of tolerant genotypes (plant adaptation to the soil) are two complementary possibilities in solving the problems related to the increase of soil productivity (Pandey et al., 2007).

### Conclusion

A great distribution of acid soils in Serbia is one of the limiting factors of the plant production. The application of certain agromelioration/pedo-meliorative measures (liming) can significantly contribute to the improvement of unfavourable, chemical properties, i.e. contribute to neutralisation of the soil altered acidity, availability of certain nutrients, then to the increase of microbiological activity and establishing the air and water regime of the soil. We presume that ameliorative fertilization in combination with liming could be better solution for soil and crop management practice with aspect of yield increases of field crops. Choice of maize hybrids for soils of moderate fertility based on the field experiments is rather useful for improvement of cropping technology and stability of maize yield.

### Acknowledgements

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## RELATIONSHIP BETWEEN SOYBEAN VARIETIES, RHIZOBIA INOCULATION AND SPAD-502 CHLOROPHYLL METER READINGS IN WESTERN SIBERIA

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### Abstract

Due to climate change and an increasing demand for food and fodder, the production of soybeans (*Glycine max*) in Russia as well as in Western Siberia increases. Within the framework of the interdisciplinary German-Russian project SASCHA a field trial was installed in Kuchak, Western Siberia (Russian Federation) in 2013 to investigate the relationship between soybean varieties, rhizobia inoculation and SPAD-502 chlorophyll meter readings. Within two German and one Siberian variety SPAD values were measured as well as numbers of nodules counted. The plants were grown in two variants (with and without inoculation) in a completely randomized block design with four replications. Only the inoculated varieties developed nodules. The German varieties showed significant higher SPAD meter readings at the latest measurement during mid of seed development. Due to low soil temperatures differences may not have occurred earlier.

**Keywords:** Soybean, inoculation, nodulation, chlorophyll content, nitrogen fixation

### Introduction

Due to climate change and an increasing demand for food and fodder, the production of soybeans (*Glycine max*) in Russia as well as in Western Siberia increases (Kiselev et al., 2013; Faostat, 2011). As a legume crop species soybeans do have a close relationship between the availability of nitrogen either from N<sub>2</sub>-fixation or soil N and the net leaf photosynthetic rate (Vollmann et al., 2011). Biological nitrogen fixation requires necessarily the inoculation with specific rhizobia bacteria e.g. *Bradyrhizobium japonicum* (Werner, 1999). Soils usually lack *Bradyrhizobium japonicum* strains (Hiltbold et al., 1985). Therefore it is important to inoculate seeds especially if soy is planted on the field for the first time, otherwise no nodulation will occur (Solomon et al., 2012). High variabilities in nodulation (Cregan et al., 1989) and N<sub>2</sub> fixation (Herridge and Rose, 2000) between soybean genotypes are reported. By comparing the leaf chlorophyll content of plants with and without nodules it is possible to quantify how much inoculation may increase the N-uptake (Vollmann et al., 2011). Leaf chlorophyll metering e.g. by Minolta SPAD-502 appears to be a useful technique for determining the nitrogen status of soybeans (Gwata et al., 2004).

Within the framework of the interdisciplinary German-Russian project SASCHA (SASCHA, 2013) a field trial was installed to investigate the relationship between soybean varieties, rhizobia inoculation and SPAD-502 chlorophyll meter readings. The results were compared with a similar trial which took place in Osnabrueck, Northern Germany in 2011.

## Materials and methods

A field trial with soybean varieties from Germany and Siberia was established at the experimental site “Kuchak” of Tyumen State University in Western Siberia (57°20'56"N, 66°3'24"E) in May 2013 (Figure 1).



Figure 1: Experimental station „Kuchak“/ Tyumen State University in Tjumen/Western Siberia

Kuchak shows continental climate with a mean min/max temperature of  $-2.9/6.9^{\circ}\text{C}$  (range from  $-42$  to  $38^{\circ}\text{C}$ ) and yearly average precipitation of 450 mm. The experimental site (podsolic soil) was irrigated during the trials as required.

Two early/very early varieties from Germany (Augusta, Aveline) and one regional Siberian (Sibniik315) were grown in completely randomized block design with four repetitions in two variants: with and without *Bradyrhizobium japonicum* inoculation. The treatment was done directly before seeding (15.05.2013) with the peat based two components product “Force48”.

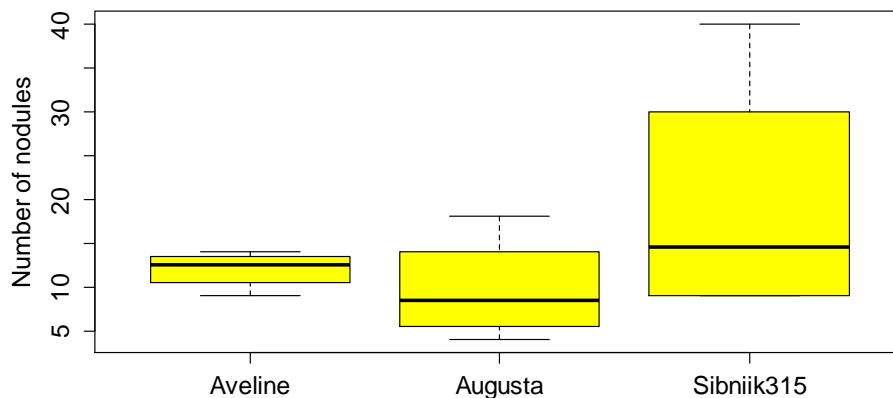
Chlorophyll content was measured at the last full developed leaf using a Minolta SPAD-502 device as average value from 30 measurements per plot. The relationship between SPAD values and chlorophyll content is non-linear for soybeans and follows an exponential equation (Markwell et al., 1995). SPAD meter readings as well as leaf chlorophyll content is closely related to leaf nitrogen content (Fritschi and Ray, 2007).

Numbers of nodules were counted on carefully digged out roots at randomly collected plants from each plot.

## Results and discussion

The number of nodules has shown significant differences between inoculated and control among all varieties. No plant of the control group was infected by bacterias, but at every inoculated plant nodules developed (Figure 2). At roots from the regional Siberian variety the highest number of nodules was counted. End of July (full flowering) soy plants from Aveline had from 9 to 14, those from Augusta between 4 and 18 while Sibniik315 showed 9 to 40 nodules.





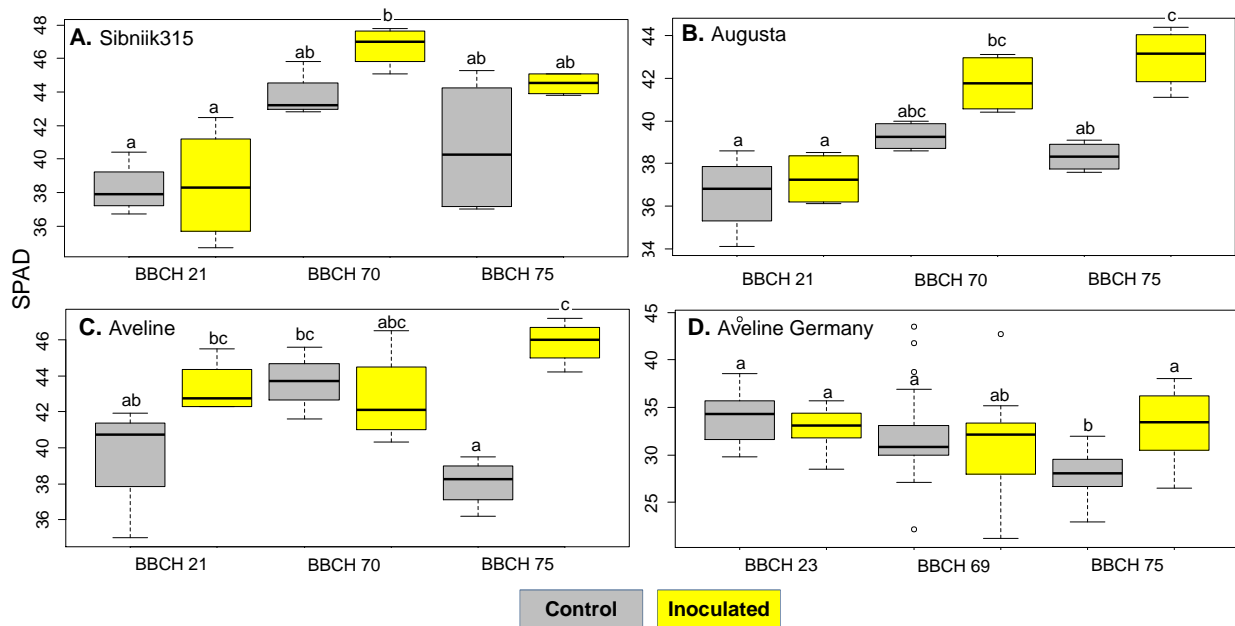
**Figure 2:** Number of nodules at inoculated varieties from Germany (Aveline, Augusta) and Siberia (Sibniik315) at full flowering (BBCH 65) on July 20<sup>th</sup> 2013.

At all plants the nodules were located close to the main root with diameters between 3-5 mm at that stage of development (Figure 3B). The color of the nodules was light red in the inside which means that they were slightly active (Figure 3C).



**Figure 3:** Roots from two Sibniik315 plants without (A) and with (B) inoculation, sliced nodule (C) during full flowering (BBCH 65).

According to the amount of nodules among the inoculated variants also the SPAD-502 chlorophyll meter readings showed differences. SPAD values tended to be higher with inoculation than in the untreated control group (Figure 4). Significant differences appeared at the third measurement (latest observed stage) during mid of seed development. In average Aveline showed the highest increase in SPAD values as well in Siberia (+20.3%) as in Germany (+19.1%) compared to control, followed by Augusta (+12.7%) and Sibniik315 (+9.8%) in Siberia.



**Figure 4:** SPAD meter readings from 3 soybean varieties in Siberia (A,B,C) and Germany (D) at beginning of side shoot formation (BBCH 21/23), end of flowering (BBCH 69/70) and mid of seed development (BBCH 75).

Comparable with the result reported by (Herridge and Rose, 2000) the development of leaf chlorophyll content was specific for every variety. No significant differences among inoculated plants at all three observations could be measured in Aveline at both locations (Figure 4 C,D yellow). In contrast SPAD-values of inoculated Augusta and Sibniik315 increased significant over time (BBCH 21-75), but no significant differences occur without treatment (Figure 4 A,B).

The highest Sibniik315 SPAD value was noticed at the second measurement (BBCH 70, inoculated) which decreased slightly in BBCH 75 (Figure 4 A) as well as in the control. This may imply a better adaptation of the Siberian variety to the local climate condition (short vegetation period) than the German breedings because relocation effects from vegetative to generative plant organs occur earlier. Nevertheless inoculated Sibniik315 showed the highest number of nodules, SPAD measurements were less distinctive (not significant) higher compared to control.

In addition to the short vegetation period in Siberia also soil temperatures play an important role for plant physiological processes of nodulating legumes.

**Table 1:** Soil temperature in 12-20cm depth. Average of 3 measurements  $\pm$  standard derivation

Date [dd/mm/yy]	BBCH	Soiltemperature [ $^{\circ}$ C]
12/05/13	before seeding	10.0 $\pm$ 1.0
28/05/13	09	14.6 $\pm$ 1.1
19/06/13	13	21.3 $\pm$ 2.5
08/08/13	74	20.0 $\pm$ 1.0

Optimal conditions for symbiotic fixation are soil temperatures between 25 to 30 $^{\circ}$ C (Lynch and Smith, 1993; Zhang et al., 1996). In contrast the measured soil temperatures in Kuchak were only  $\pm$  20 $^{\circ}$ C until BBCH 74 (Table 1) and may cause the slow increase of symbiotic activity.

## Conclusion

Inoculation of soybeans with *Bradyrhizobium japonicum* is necessary otherwise no nodules for atmospheric nitrogen fixation will be developed under Siberian conditions. Due to low soil temperatures below the optimal range for symbiotic activity the significant effect of inoculation shows up at a late stage of development (BBCH 75). Therefore the advantages of inoculation may be limited by Siberian climate conditions.

Local varieties are better adapted to local conditions, but genotypes from other regions must be used to improve the local breedings.

After harvesting and analyzing the varieties in October final conclusions will be possible.

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## IN SITU BREEDING FOR SOIL ABIOTIC STRESS TOLERANCE IN WHEAT

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Plant breeding programs are mainly settled in breeding institutions accompanied by experimental fields established nearby. Experimental fields are usually organized in favorable agro-technical conditions on a soil as best as possible. That is understandable, because the targets of breeding process, as well, as selection criteria are adjusted for intensive agricultural conditions. However, the increasing demand for food requires all the available resources to be put in good use. In that aspect, the land non-suitable for agricultural production, at the moment, become of certain interest for broadening agricultural potentials. More intensive agricultural use of less productive or degraded soil, involves novel genotypes obtained under adequate selection criteria in specialized breeding programs. The general debate is whether *ex situ* breeding programs were good enough to meet the challenge or *in situ* established breeding would be required? Experiences and results from parallel wheat breeding trials on chernozem, and solonetz soil are commented in the article.

**Key words:** wheat, breeding, abiotic stress, soil

### Introduction

Wheat represents, in various forms, an essential food in the Western civilization. The run for the food production enhancement and agricultural industrialization started with the Green revolution in a second half of the past century. All the achievement in wheat breeding was sublimated in 1970, when Norman Borlaug was awarded the Nobel Peace Prize. The advent of semi-dwarf germplasm created primarily by Nazareno Strampelli in 1930s, as well as, considerable later by Norman Borlaug in CIMMYT wheat breeding program in the late 1960s, had a great impact on agriculture (Salvi et al., 2013., Phillips, 2013.). According to World Bank estimation, thirty years ago half of the developing world lived in extreme poverty. Today, that is a quarter. In today world, children malnutrition, and a risk of early death is quite diminished (World bank, 2010.). Whole wheat plant architecture has been changed, in order to ensure food security that has been a major challenge in 20th Century, and is going to be even a greater challenge in time to come. Due to globalization, and market integration, bread wheat is preferred over rice, sorghum and millet-based foods, in developing countries (Wrigley, 2009.). Furthermore, global food demand will increase, along with the population size increment. According to projection, 8 billion by 2030, and probably over 9 billion people by 2050 are going to put a heavy pressure on the global food system over the next 40 years (Foresight, 2011.).

There are, generally, two ways to responding to food production challenge. One is through wheat plant ideotype changes. In 1960s Donald (1968) appeared with wheat plant ideotype with improved sink to source ratio (harvest index) that was able to give higher grain yield due to more efficient assimilative translocation from vegetative to generative part of the plant. In the past 50 years the semi-dwarf plant stature (60-80cm), semi-erect leaves, large ear, harvest index of 50-60% was predominant in a wheat production. That kind of plant architecture has the yield potential above 10t/ha (Borojevi , 1983.). However, to make a run for the new border of 20t/ha, wheat breeders have to make a qualitative step forward by establishing a new ideotype. Den i (1994) reported promising effort that has been made for

ten years to enhance sink capacity by creating branched and tetrastichon (two spikelets per node of the rachis) type of spike. Other researchers investigated the improvement of source capacity by changes in flag leaf angle, anatomy, chlorophyll content (Liu et al., 2009.). The other way is to enhance food production by converting non-arable to arable land, as well as, to put less productive soil in more intensive agricultural use. From the beginning of 1960s, the growth in food production has been based on genotype and agricultural practice improvements, while agricultural land mass stayed almost at the same level (fig. 1).

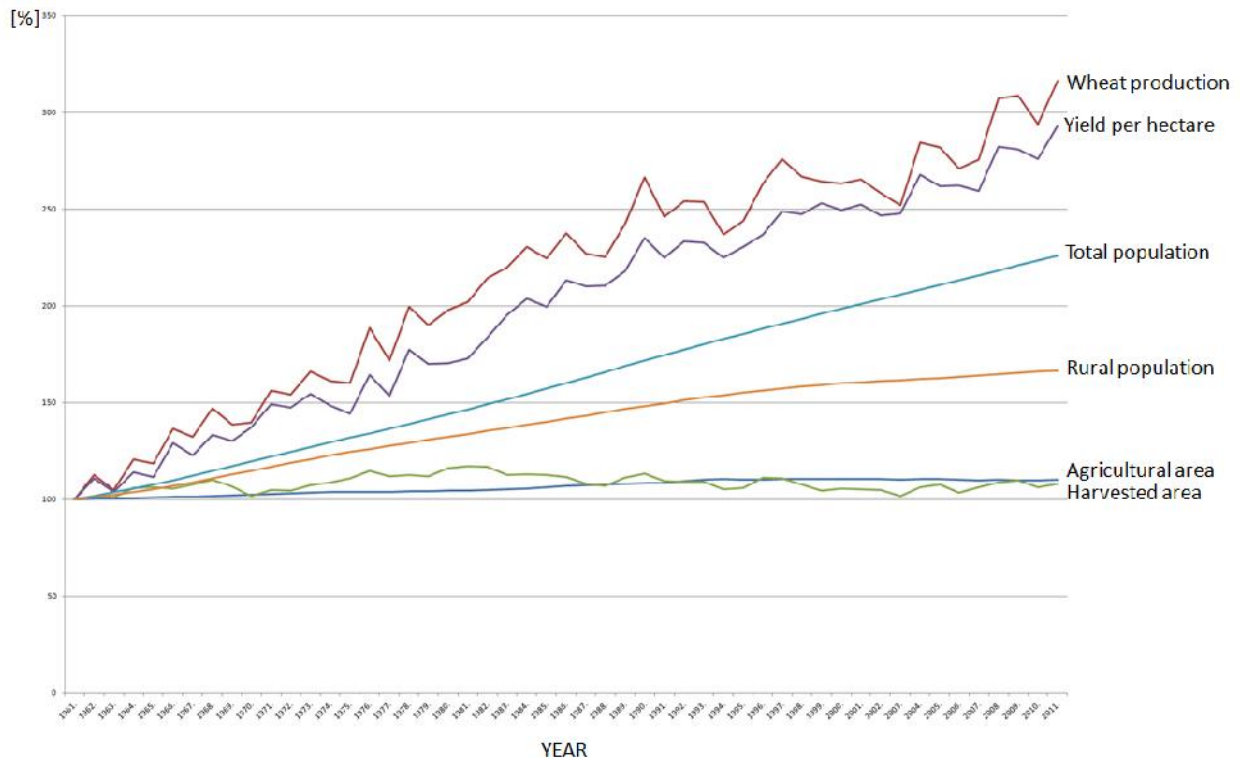


Figure 1. Agricultural area vs. wheat production and human population from 1961 (indexed 100) to 2011. (Data source: FAO)

Less productive soil could be an interesting source of broadening agricultural area by using suitable crop and ameliorative measures. Solonetz soil occupies about 1% of the land in the World, and about 0.5% in Europe. (Encyclopedia Britannica, 2013.). That soil type is characterized by alkaline pH reaction, sodium accumulation, and strong clay sub arable layer. In natural form, commonly occurs in dry, flat continental areas and could be used as a pasture. In Serbia, solonetz covers about 80000ha in the northern part of the country in Banat, mostly (Beli , 1999).

The aim of the article is to estimate the possibility of utilizing solonetz for wheat production, and to investigate the possibilities of creating novel genetic variability acceptable adapted to solonetz abiotic stress conditions in an *in situ* established wheat breeding program.

## Materials and methods

Five parental varieties of bread wheat (*Triticum aestivum ssp. vulgare* L.), Pobeda, Renesansa, Sara, Partizanka and Pesma, and seven of their F<sub>2</sub> progenies, from an *in situ* established wheat breeding program, were studied. The experiment has been conducted in vegetation periods (2009/2010, 2010/2011, and 2011/2012), at two localities Kumane village (45.521994<sup>0</sup>N, 20.194919<sup>0</sup>E, pH = 9.86) on solonetz soil, and Rimski Šan evi experimental field of the Institute of Field and Vegetable Crops in Novi Sad (45.324936<sup>0</sup>N, 19.842883<sup>0</sup>E, pH=6.86) on chernozem, parallel. All the wheat cultivars have been created at the Institute of Field and Vegetable Crops in Novi Sad. Three wheat plant traits were followed: plant height (cm), grain number and grain weight (g) per spike. According to the results of Denić *et al.* (2000), individual plant traits are closely correlated to grain yield in abiotic stress conditions. Moreover, phenotypic expression of individual plant traits is the only usable selection criteria for desirable genotype selection in F<sub>2</sub>. Each variety, as well as, progeny was sown, spike per row, in eight 12.5cm spaced rows one meter long with 20cm space between plots. Fifty kilograms of NPK 15:15:15 fertilizer was applied along with sowing, and 150kg of KAN was additionally applied in spring. The whole *in situ* breeding program covers about 2ha. Elementary statistics has been calculated for quantifying and describing studied traits. The mode of inheritance has been established using t-test.

## Results and discussion

The specific soil conditions of solonetz could be considered as abiotically stressful environment (Dimitrijević *et al.*, 2011). The cultivar of choice for that surrounding should fulfill the following criteria: to be durable, to have at least moderate tolerance to salinity, to be of higher economic interest, to be of low input production and to be usable as ameliorator of the soil. Wheat (*Triticum aestivum*) is a moderately salt-tolerant of low input production crop, as well as, essential and valuable source of nutrition providing social security for the farmer and society (Farooq and Azam, 2001, Munns *et al.*, 2006). After the crop selection, the next step was to test the existing and available genetic variability (Petrović *et al.*, 2010). During a decade of investigation several wheat varieties have been selected as promising parents in crosses (Dimitrijević *et al.*, 2009, Petrović *et al.*, 2009). A parallel breeding program has been established on solonetz (*in situ*) and chernozem (*ex situ*). Having two part of the experiment geographically close, environmental variation due to meteorological conditions is considerable diminished. The same goes for agricultural practice which was the same, as well as, genetic material in study. That way the main differences are caused by soil variation. The following results illustrate advantages and disadvantages of an *in situ* breeding program for abiotic stress tolerance that is halomorphic soil in this experiment. According to previous results, selection criteria have been built. Since, F<sub>2</sub> is compared, phenotypic markers are followed. The general approach has been to reach wheat plant habitus of normal environmental condition chernozem provides. According to the results, novel genetic variability has to compensate about 30% of plant height loss due to solonetz soil conditions. Predominant heterotic effect that appears in F<sub>2</sub> in two of three examined vegetation periods, gives a space to work in that direction in progenies that follows, and to get that heterotic effect fixed (tab.1). Overdominance that is denoted in F<sub>2</sub> progenies for the grain number and grain weight per spike looks promising for further selection that should catch up about 20-25% off solonetz to chernozem differences (tab. 2, tab. 3, respectively). The problem is that unfavorable meteorological conditions make greater

pressure on plants in solonetz soil environment, comparing to one on chernozem soil. The loss of some progenies at the locality of Kumane had been just from that reason, because weather conditions caused hard crust formation on the surface of solonetz soil, but not on chernozem (tab. 1, tab. 2, and tab. 3). However, hush conditions of solonetz in an *in situ* breeding program put the genetic material in a real situation. Phenotypic variability appears not only because of soil type, but also due to complex genotype by environment interactions that are quite different in abiotic stress, comparing to normal growth conditions. Unfavorable conditions generally diminish the cultivar differences and enhance genotype by environment interaction.

Table 1. Mean values of number of plant height (cm) on black soil ( $\bar{x}_{Ch}$ ) and solonetz ( $\bar{x}_{So}$ ) over three wheat growing seasons. The differences ( $d$ ) and environmental means ( $\bar{X}_E$ ) are given

Year	2009/2010			2010/2011			2011/2012		
	$\bar{x}_{Ch}$	$\bar{x}_{So}$	$d$ (%)	$\bar{x}_{Ch}$	$\bar{x}_{So}$	$d$ (%)	$\bar{x}_{Ch}$	$\bar{x}_{So}$	$d$ (%)
<i>Parents</i>									
Pobeda (P1)	83.7	55.6	67	73.3	61.0	83	65.9	71.6	109
Renesansa (P2)	87.7	59.0	67	76.3	61.8	81	67.4	67.7	100
Sara (P3)	87.2	51.3	59	72.8	61.3	84	64.2	57.1	89
Partizanka (P4)	90.5	62.9	70	79.0	64.1	81	68.2	60.0	88
Pesma (P5)	88.4	54.4	62	83.5	58.1	70	63.0	73.3	116
$\bar{X}_E$	87.5	56.7	65	77.0	61.2	80	65.7	65.9	100
<i>F<sub>2</sub> progenies</i>									
P1 X P3	<sup>+h</sup> 107.2	<sup>+h</sup> 79.6	74	<sup>+h</sup> 85.8	<sup>+h</sup> 91.2	106	<sup>+d</sup> 67.2	-	-
P1 X P5	<sup>+h</sup> 96.2	<sup>+h</sup> 80.5	84	<sup>-d</sup> 76.5	<sup>+h</sup> 85.1	111	<sup>-d</sup> 63.7	-	-
P2 X P3	<sup>+h</sup> 93.5	<sup>+h</sup> 76.3	82	<sup>-d</sup> 73.3	<sup>+h</sup> 76.4	104	<sup>+d</sup> 68.1	-	-
P2 X P5	<sup>-</sup> 92.0	<sup>+h</sup> 78.3	85	<sup>-h</sup> 66.7	<sup>+h</sup> 80.7	121	<sup>-d</sup> 63.1	<sup>+d</sup> 71.3	113
P3 X P4	<sup>+h</sup> 100.6	<sup>+h</sup> 70.9	71	<sup>+h</sup> 91.6	<sup>+h</sup> 91.5	100	<sup>+h</sup> 78.0	<sup>+d</sup> 71.2	91
P3 X P5	<sup>-</sup> 90.9	<sup>+h</sup> 76.0	84	<sup>-d</sup> 76.4	<sup>+h</sup> 88.6	115	<sup>+h</sup> 70.6	<sup>+h</sup> 71.3	101
P4 X P5	<sup>+d</sup> 92.7	<sup>+h</sup> 82.9	89	<sup>-</sup> 78.5	<sup>+h</sup> 88.3	112	<sup>+d</sup> 67.8	<sup>+d</sup> 71.6	106
$\bar{X}_E$	94.5	78.1	83	78.4	86.0	110	68.4	71.3	104
$\bar{X}_E$	92.0	70.4	77	77.8	75.7	98	67.0	68.6	102
LSD <sub>0.05</sub> =4.382 LSD <sub>0.01</sub> =5.320 LSD <sub>0.05</sub> =5.928 LSD <sub>0.01</sub> =7.931 LSD <sub>0.05</sub> =1.756 LSD <sub>0.01</sub> =2.312									



Table 2. Mean values of number of grain number per spike on black soil ( $\bar{x}_{Ch}$ ) and solonetz ( $\bar{x}_{So}$ ) over three wheat growing seasons. The differences ( $d$ ) and environmental means ( $\bar{X}_E$ ) are given

Year	2009/2010			2010/2011			2011/2012		
	$\bar{x}_{Ch}$	$\bar{x}_{So}$	$d$ (%)	$\bar{x}_{Ch}$	$\bar{x}_{So}$	$d$ (%)	$\bar{x}_{Ch}$	$\bar{x}_{So}$	$d$ (%)
<i>Parents</i>									
Pobeda (P1)	43	29	68	45	30	66	33	37	112
Renesansa (P2)	47	32	67	44	31	70	30	43	145
Sara (P3)	45	34	76	37	36	99	37	34	90
Partizanka (P4)	38	34	87	36	34	95	30	28	94
Pesma (P5)	46	39	84	37	36	98	32	42	130
$\bar{X}_E$	44	34	76	40	33	84	32	37	113
<i>F<sub>2</sub> progenies</i>									
P1 X P3	<sup>-</sup> 42	<sup>+h</sup> 44	106	<sup>-d</sup> 37	<sup>+h</sup> 46	126	<sup>-d</sup> 31	-	92
P1 X P5	<sup>-h</sup> 36	<sup>+d</sup> 40	112	<sup>-h</sup> 36	<sup>+h</sup> 49	134	<sup>+h</sup> 38	-	104
P2 X P3	<sup>-</sup> 44	<sup>+d</sup> 40	92	<sup>-d</sup> 39	<sup>+h</sup> 45	117	<sup>+h</sup> 39	-	105
P2 X P5	<sup>-</sup> 50	<sup>+h</sup> 47	94	<sup>-</sup> 40	<sup>+d</sup> 37	91	<sup>+d</sup> 33	<sup>-h</sup> 30	104
P3 X P4	<sup>-</sup> 42	<sup>+h</sup> 45	108	<sup>-</sup> 36	<sup>+h</sup> 41	114	<sup>-h</sup> 30	<sup>-h</sup> 31	95
P3 X P5	<sup>-</sup> 47	<sup>-</sup> 40	85	<sup>+h</sup> 45	<sup>-</sup> 40	90	<sup>-d</sup> 31	<sup>-d</sup> 32	104
P4 X P5	<sup>-</sup> 43	<sup>-</sup> 37	86	<sup>-</sup> 35	<sup>+h</sup> 48	136	<sup>-d</sup> 32	<sup>-d</sup> 33	92
$\bar{X}_E$	44	43	97	38	44	114	34	32	104
$\bar{X}_E$	44	40	90	39	39	101	33	34	105
LSD <sub>0.05</sub> =5.354    LSD <sub>0.01</sub> =7.121    LSD <sub>0.05</sub> =4.268    LSD <sub>0.01</sub> =5.71    LSD <sub>0.05</sub> =1.505    LSD <sub>0.01</sub> =1.981									

Table 3. Mean values of number of grain weight per spike (g) on black soil ( $\bar{x}_{Ch}$ ) and solonetz ( $\bar{x}_{So}$ ) over three wheat growing seasons. The differences ( $d$ ) and environmental means ( $\bar{X}_E$ ) are given

Year	2009/2010			2010/2011			2011/2012		
	$\bar{x}_{Ch}$	$\bar{x}_{So}$	$d$ (%)	$\bar{x}_{Ch}$	$\bar{x}_{So}$	$d$ (%)	$\bar{x}_{Ch}$	$\bar{x}_{So}$	$d$ (%)
<i>Parents</i>									
Pobeda (P1)	1.70	1.22	72	2.05	1.31	64	1.7	1.6	97
Renesansa (P2)	1.83	1.37	75	1.89	1.31	69	1.9	1.9	99
Sara (P3)	1.65	1.13	68	1.60	1.49	93	1.8	1.1	63
Partizanka (P4)	1.42	1.33	94	1.77	1.31	74	1.4	0.7	55
Pesma (P5)	1.68	1.32	79	1.59	1.43	90	1.2	1.5	122
$\bar{X}_E$	1.66	1.27	76	1.78	1.37	77	1.6	1.4	86
<i>F<sub>2</sub> progenies</i>									
P1 X P3	<sup>-</sup> 1.58	<sup>+h</sup> 1.75	111	<sup>+d</sup> 2.04	<sup>+h</sup> 1.97	97	<sup>-</sup> 1.7	-	-
P1 X P5	<sup>-h</sup> 1.33	<sup>-</sup> 1.56	117	<sup>-d</sup> 1.59	<sup>+h</sup> 1.99	125	<sup>+h</sup> 1.9	-	-
P2 X P3	<sup>-</sup> 1.70	<sup>-</sup> 1.49	88	<sup>-h</sup> 1.55	<sup>+h</sup> 1.85	119	<sup>+h</sup> 2.0	-	-
P2 X P5	<sup>-</sup> 1.86	<sup>+h</sup> 1.90	102	<sup>-d</sup> 1.40	<sup>-</sup> 1.53	110	<sup>-d</sup> 1.7	<sup>-h</sup> 1.1	64
P3 X P4	<sup>-d</sup> 1.78	<sup>+h</sup> 1.75	98	<sup>-</sup> 1.70	<sup>+d</sup> 1.58	93	<sup>-d</sup> 1.5	<sup>+d</sup> 1.1	74
P3 X P5	<sup>-</sup> 1.84	<sup>+h</sup> 1.71	92	<sup>+h</sup> 1.96	<sup>-</sup> 1.57	80	<sup>+d</sup> 1.7	<sup>+d</sup> 1.2	68
P4 X P5	<sup>-</sup> 1.44	<sup>-</sup> 1.47	102	<sup>-</sup> 1.59	<sup>+h</sup> 1.90	119	<sup>+d</sup> 1.4	<sup>+d</sup> 1.2	87
$\bar{X}_E$	1.67	1.69	101	1.69	1.77	105	1.7	1.1	67
$\bar{X}_E$	1.66	1.54	93	1.73	1.60	93	1.6	1.3	76.7
LSD <sub>0.05</sub> =2.256    LSD <sub>0.01</sub> =3.000    LSD <sub>0.05</sub> =0.244    LSD <sub>0.01</sub> =0.327    LSD <sub>0.05</sub> =0.126    LSD <sub>0.01</sub> =0.166									

In *ex situ* breeding program targeted for special purposes that is abiotic stress tolerance in this experiment, there is an undeniable cosines of more secure environment giving considerable certainty. Commonly, the *ex situ* breeding approach is combined with physiological or molecular studies conducted in order to find certain gene(s) for salinity tolerance, high Na<sup>+</sup> content, etc. However, in rencounter of complex and interactive genetic background and high

selection pressure of complex environmental influence of solonetz soil conditions combined with meteorological and climate change influences, the eventual satisfactory result depends on more factors than  $\text{Na}^+$  or salinity introduced tolerance could be. The experiments that include *in situ* vs. *ex situ* breeding comparison are to be continued....

### Conclusion

Comparison of *in situ* and *ex situ* wheat breeding program for getting genotypes capable to withstand hush conditions of solonetz field production, reveals some potential advantages and disadvantages of these two approaches. However, the program of genetic variability creation exhibiting higher level of tolerance to stressful growing conditions of alkaline soil in wheat breeding *in situ* gave some promising results for yield components considered in the article as phenotypic markers in early generations of crosses. Induced genetic variability obtained in an *in situ* breeding program on solonetz soil, by crossing parents selected in the previous years of experiments, open a perspective of superior genotypes selection in  $F_2$  populations. That gives the possibility of desirable variation creation by the selection in consequent progenies to obtain varieties that could give an economically sound result maximizing the use value of halomorphic, alkaline solonetz soil.

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## THE STABILITY PROPERTIES OF WHEAT PRODUCTION ON ACID SOIL

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### Abstract

The investigation was carried out on the experimental field of Center for Small Grains, Kragujevac. This paper presents the results of winter wheat varieties (Takova anka, KG 100, KG 56S, Ana Morava and Lazarica). Grain yield, 1000 kernel weight and test weight in grain the investigated wheat cultivars was determined in a two-year field experiment.

Average grain yield of wheat cultivars ranged from 2.151 t/ha to 4.206 t/ha. Grain yield differed significantly between years and the average of all cultivars was higher in 2006/07. compared to 2005/06. The study of physical properties of grain, cultivar KG 56S had the largest average grain yield in 2005/06th year (2.455 t/ha), while in the second year the highest yield was cultivar Ana Morava (4.206 t/ha). Average values of 1000 grain weight of wheat cultivars varied in the range from 36.26 to 42.58 g.

The difference found between the significance of the impact on the quality of grain and test weight of wheat cultivars were significant for grain yield. Analysis of the data revealed that the genotype is very significant impact on the 1000 grain weight.

**Key words:** 1000 grain weight, grain yield, wheat

### Introduction

Winter wheat (*Triticum aestivum* L.) is one of the most important crops in Serbia, and sown on about 530,000 ha per year. The average yield of wheat last 10 years in the major production areas of Serbia ranging from 4.5 to 8.0 t/ha. For a successful and stable wheat production is necessary synergism high-yielding varieties, optimal growing conditions, application of modern agricultural practices and plant protection. Grain yield is a complex trait that depends on the genotype and the environmental conditions in which plants are grown (Ekić et al., 2010; Đurić et al., 2012, 2013; Perišić et al. 2011). Production of wheat with high grain yield and quality is appropriate only possible choice of high-quality varieties, but with the proper growing conditions and appropriate production technologies. However, wheat quality is a debatable point, which depends on whether it is viewed from the aspect of growers or users, but in any case it calls for continual improvement (Hristov et al., 2010).

Development of new genotypes, highly adapted to particular agroecological conditions, is crucial for obtaining high-quality end products (Milovanović et al., 2011). Success in breeding depends not only on the genotype but also on environmental factors. Successful wheat breeding is based on the knowledge of characteristics of wheat genotypes, as well as their interactions with the environment (Zević et al., 2009; Ekić et al., 2010). Understanding these relationships may serve to determine the breeding strategy, to identify favorable conditions for testing, as well as to zone the new genotypes for the different agroecological conditions.

Vegetation during the year (2005-2007) in field trials in the grounds of the Center for Small Grains, Kragujevac examined the five varieties of winter wheat, with the aim of determining the selection of the best varieties for the production requirements Serbia.

### Materials and methods

#### *Materials and field trials*

During the 2005/06 and 2006/07 growing seasons, five cultivars of winter wheat were investigated, cultivated at the Center for Small Grains in Kragujevac. The cultivars Takov anka, KG 100, KG 56S, Ana Morava and Lazarica had been investigated. Experiments have been conducted in randomized block systems, with a plot size of 10 m<sup>2</sup> in five replicates. The usual techniques for wheat production were applied, and it was done in the optimum sowing time in late October. 80 kg/ha N, 100 kg/ha P<sub>2</sub>O<sub>5</sub> i 60 kg/ha K<sub>2</sub>O of fertilizer NPK 8:24:16 was added in the fall on the investigated plots, while during the spring fertilization, AN (17% N) was supplemented.

The crop was harvested at full maturity. Grain yield (t/ha) was harvested and reported at 14% moisture. Two parameters of grain quality, namely test weight (kg/hl) and 1000-grain weight (g) were analyzed. Thousand grain weight was determined using an automatic seed counter. Test weight is the weight of a measured volume of grain expressed in kilograms per hectoliter.

On the basis of achieved research results the usual variational statistical indicators were calculated: average values, error of the mean (arithmetic) and standard deviation. Statistical analysis was made in the module Analyst Program SAS/STAT (SAS Institute, 2000.).

#### *Soil and weather conditions*

Before the commencement of the experiment soil samples were taken from the sample surface and the chemical analysis of soil was performed. On the basis of obtained results it was revealed that the soil belongs to the vertisol soil type, with relatively high clay content, and unfavorable physical properties. The humus content in the surface layer of soil was low (2.22%), and a substitution and total hydrolytic acidity were quite high (pH H<sub>2</sub>O=5.39, KCl=4.43). The soil was medium provided with total nitrogen (0.11-0.13% N) and easily accessible potassium (10-14 mg/100 g soil K<sub>2</sub>O), while the available phosphorus content was low (under 10 mg/100 g of soil P<sub>2</sub>O<sub>5</sub>).

**Table 1** Middle monthly air temperature and precipitation amount (Kragujevac)

Year	Months												Aver.
	VIII	IX	X	XI	XII	I	II	III	IV	V	VI	VII	
Mean monthly air temperature (°C)													
2005-06	20.0	17.4	11.5	5.7	3.4	1.4	-1.7	4.7	11.6	16.5	19.3	21.7	11.0
2006-07	20.7	17.7	13.3	7.6	3.5	-1.7	1.5	5.6	12.7	16.4	19.7	23.0	11.7
Average 1961-90	21.0	16.9	11.8	5.6	1.9	0.6	2.0	6.2	11.2	16.2	19.4	21.3	11.2
The amount of rainfall (mm)													
2005-06	117.8	115.6	49.0	54.8	47.9	36.6	66.9	44.5	69.0	70.2	50.8	86.2	809.3
2006-07	141.9	57.4	16.7	13.7	51.9	27.9	38.1	116.1	86.3	29.6	84.8	22.4	686.8
Average 1961-90	49.1	42.0	47.5	50.0	49.5	36.8	33.9	43.5	51.5	64.8	79.3	62.5	610.4

Kragujevac area is characterized by a moderate continental climate, which general feature is uneven distribution of rainfall by month. The data in Table 1 for the investigated period (2005-2007) clearly indicate that the years in which the researches were conducted differed from the typical multi-year average of Kragujevac region regard the meteorological conditions. The average air temperature was 0.2°C lower in 2005/06 and 0.5°C higher in 2006/07, as compared to the long-term mean, whereas the sum of rainfall was 198.9 mm and 76.4 mm higher in respective years as compared to the long-term mean. Spring months April, May and June 2005/06 were with overly precipitation, which affected unfavorable on the crops. During the Mart in 2006/07 it was 116.1 mm of rainfall, what was 83.7 mm more compared with the perennial average. In May of 2005/06 it was 70.2 mm of rainfall, what was 12.6 mm more compared with the perennial average.

### Results and discussion

Based on the analysis of variance, it can be concluded that there are very significant differences in grain yield regard the year of investigation ( $F_{\text{exp}}=31.365^{**}$ ) and very significant differences at grain test weight, relative while among the investigated wheat cultivars the differences were not significant. Very significant differences in 1000 grain weight at investigated wheat cultivars were found relative to the cultivar. In the dual interaction between the cultivar and year, significant effects were noted on grain test weight (Table 2). Our results are consistent with the results eki et al. (2010, 2012) and Jeli et al. (2013), where the authors state that the growing conditions in the observed years had a significant impact on yield.

**Table 2** Analysis of variance of the traits of wheat (ANOVA)

Effect of year on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(df1.2)	p-level
Grain yield (t/ha)	24.5532	0.782830	31.365 <sup>**</sup>	0.000001
1000 grain weight (g)	10.3968	5.358917	1.940	0.170076
Test weight (kg hl <sup>-1</sup> )	291.3698	9.497504	30.679 <sup>**</sup>	0.000001
Effect of cultivars on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(df1.2)	p-level
Grain yield (t/ha)	0.58292	1.32883	0.439	0.779965
1000 grain weight (g)	35.91220	2.75502	13.035 <sup>**</sup>	0.000000
Test weight (kg hl <sup>-1</sup> )	22.77950	14.58071	1.562	0.200728
Effect of the year x cultivars interaction on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(df1.2)	p-level
Grain yield (t/ha)	0.92629	0.788475	1.175	0.336462
1000 grain weight (g)	4.60180	2.379300	1.934	0.123511
Test weight (kg hl <sup>-1</sup> )	22.08630	6.910425	3.196 <sup>*</sup>	0.022793

\*Statistically significant difference ( $P<0.05$ ) \*\*Statisticaly high significant difference ( $P<0.01$ )

The grain yield of wheat significantly varied across years, from 2.313 t/ha in 2005/06 to 3.715 t/ha in 2006/07 (Table 3). Average grains yield observed in the two-year period was the highest at Ana Morava cultivar (3.229 t/ha), while the lowest yield was obtained by KG 100

cultivar (2.681 t/ha). The average grains yield over a two-year period was 3.014 t/ha. During the first year of investigations, cultivar KG 56S achieved the highest grains yield (2.455 t/ha), followed by Lazarica (2.404 t/ha), while the lowest yield was at Takov anka cultivar (2.151 t/ha). During the second year of investigations, the yield of Ana Morava cultivar was the highest with 4.206 t/ha, while the slightly lower yield was realized by Takov anka cultivar (4.123 t/ha).

The sufficient amounts of rainfall during the spring months (Table 1), particularly April, which are vital to successful wheat production, suggest that the distribution and total amount of rainfall during the growing season 2006/07 were considerably more favorable, resulting in an increased yield in this year as compared to the first year. The significantly lower yield in 2005/06 was due to a decline in total rainfall in the spring and its non-uniform distribution across months, accompanied by higher average air temperatures in these years. eki et al. (2012) in his research states that the air temperatures and the rainfall amount and distribution during the wheat growing season have the greatest impact on high yields and grain quality. Considerable variation in grain yield on years depending of research have established eki et al. (2010), uri et al. (2012, 2013) and Periši et al. (2011).

**Table 3** Average values of the traits of wheat

Cultivars	2005/06			2006/07			Average		
	$\bar{x}$	S	$S_x$	$\bar{x}$	S	$S_x$	$\bar{x}$	S	$S_x$
Grain yield (t ha <sup>-1</sup> )									
Takov anka	2.151	0.944	0.422	4.123	1.086	0.486	3.137	1.414	0.447
KG 100	2.304	0.814	0.364	3.058	0.996	0.445	2.681	0.945	0.299
KG 56S	2.455	0.686	0.307	3.220	0.809	0.362	2.838	0.814	0.257
Ana Morava	2.253	0.685	0.306	4.206	1.042	0.466	3.229	1.323	0.418
Lazarica	2.404	0.742	0.332	3.969	0.965	0.431	3.186	1.157	0.366
Average	2.313	0.721	0.144	3.715	1.023	0.205	3.014	1.126	0.159
1000 grain weight (g)									
Takov anka	38.84	0.744	0.333	39.34	1.744	0.780	39.09	1.291	0.408
KG 100	38.98	0.726	0.325	37.08	2.051	0.917	38.03	1.763	0.557
KG 56S	42.12	1.746	0.781	42.58	2.314	1.035	42.35	1.948	0.616
Ana Morava	39.34	1.113	0.497	38.20	1.679	0.751	38.77	1.471	0.465
Lazarica	38.74	1.043	0.466	36.26	1.383	0.618	37.50	1.744	0.552
Average	39.60	1.663	0.333	38.69	2.820	0.564	39.15	2.337	0.330
Test weight (kg hl <sup>-1</sup> )									
Takov anka	70.61	2.920	1.306	76.79	3.871	1.731	73.70	4.589	1.451
KG 100	66.91	1.684	0.753	74.45	1.960	0.876	70.68	4.331	1.370
KG 56S	71.41	2.128	0.952	77.75	1.952	0.873	74.58	3.856	1.219
Ana Morava	72.93	1.659	0.742	72.93	4.170	1.865	72.93	2.992	0.946
Lazarica	69.97	2.198	0.983	74.05	2.366	1.058	72.01	3.043	0.962
Average	70.37	2.836	0.567	75.19	3.309	0.662	72.78	3.905	0.552

Thousand grain weight in the test period was highest in 2005/06 (39.60 g), but decreased in 2006/07 by 0.91 g or 2.30%. Cultivars had a very significant effect on the 1000-grain weight ( $p < 0.01$ ). The wheat Lazarica cultivar achieved the lowest average 1000 grain weight during the both years of investigation (38.74 g and 36.26 g) compared with other tested wheat cultivars. During the both years of investigation the highest average value of 1000 grain weight achieved the KG 56S cultivar (42.12 g and 42.58 g). A number of authors ( eki et

al. 2010, 2012; uri et al. 2012; Jeli et al. 2013) underline that 1000-grain weight is a cultivar-specific trait, with considerably higher variations being observed among genotypes than among treatments or environmental factors.

Test weight is an indicator of grain quality, particularly grain monetary value. Wheat grains having a higher test weight are generally considered to be of higher quality than those with a low test weight. During the first year achieved the highest test weight at Ana Morava cultivar (72.93 kg/hl), followed by KG 56S (71.41 kg/hl), while the lowest test weight was at KG 100 cultivar (66.91 kg/hl). During the second year of investigations, the test weight of KG 56S cultivar was the highest with 77.75 kg/hl, while the slightly lower test weight was realized by Takov anka cultivar (76.79 kg/hl). The average two-year value of test weight at KG 56S cultivar was (74.58 kg/hl), while the lowest average two-year value was at KG 100 cultivar (70.68 kg/hl). Grain of investigated wheat cultivars was characterized by good physical characteristics; especially regard the test weight and 1000 grain weight. Realized average values of these characteristics in the study were slightly lower than the values obtained by eki et al. (2010, 2012).

### Conclusion

Environmental conditions (weather and soil) have a significant effect on grain yield and quality in wheat. Grain yield shows a tendency to increase in the years having a higher total amount and better distribution of rainfall during critical plant development stages.

Based on the gain results during two-year investigation on five Kragujevac's wheat cultivars, it can be concluded that the highest yield achieved the cultivar Ana Morava. Takov anka, KG 56S and Lazarica cultivars have achieved satisfactory results, while the poorest results were achieved by the cultivar KG 100.

Considering the average yields value and test weight in 2005/06 and 2006/07, it was evidently that the yields and test weight were highly statistically significantly different between the year ( $p < 0.01$ ). Interaction of the year and cultivars on the test weight were statistically significantly. Investigations on genotype and environment interaction present the basis for further refinement and wheat zoning.

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## PRODUCTION OF ANNUAL CARAWAY IN SERBIA

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### Abstract

Caraway (*Carum carvi* L.) is an aromatic herb of the Apiaceae family. It has two forms: a biennial, which is grown in the colder and humid climates, and annual that is grown in temperate climate. In Serbia is mostly cultivated biennial caraway, which in the first year formed only a leaf rosette, and with the end of the growing season above-ground parts dies and the roots over winter and in the spring of the second year of plant development begins early. After four to five weeks from appearing of first leaves, it started growth of flowering stems. Lack of cultivation of these forms of caraway is that in the first year does not provide benefits but requires investment. Unlike the biennial forms, vegetation period of annual caraway lasts from 140-160 days, it forming a rosette with less leaves, and very quickly starts to develop flowering stems. For this reason, the aim of our study was to investigate the possibility of growing annual caraway in condition of Serbia.

Trials were conducted at three locations in Serbia, during 2011 and 2012. Sowing was carried out during April to row spacing of 35 cm, after germination of plants, thinning plants was carried to obtain 70 plants per meter. From our research, it can be concluded that the yield of annual caraway, weather conditions have a statistically significant impact. In a favorable year (2011) has been achieved the average yield of 850 kg ha<sup>-1</sup>, while in the dry year yield was very reduced (193 kg ha<sup>-1</sup>).

**Keywords:** annual caraway, yield, weather conditions

### Introduction

Caraway (*Carum carvi* L.) has been grown as a spice plant for many centuries and is almost cosmopolitan. It is native to Europe, parts of Asia and northern Africa, but it is grown also in the northern US and Canada. Caraway has two forms: biennial which is grown mainly in the northern parts of the world (Europe and America), and annual which is grown commercially in temperate zones (Mediterranean, Africa, South America and Asia).

Caraway seed has been mainly used as a spice or rather like essential oil. The seed essential oil has been used as a fragrance and flavour in the food, drinks and alcoholic beverages, perfumery and pharmaceutical industries, and for medicinal purposes. Beside essential oil, caraway seed also contain lipid (13-21%), nitrogenous compounds (25-35%), fiber (13-19%) and water (13-19%) (Gwari et al., 2012).

In Serbia, mostly is grown biennial caraway type, which in first year forming only a leaf rosette with 7-18 leaves. With the first frosts, above-ground parts die, and early in the spring next year, with increasing temperature vegetation started. After four to five weeks from the appearing of first leaves it started growth of flowering stems. In the case of biennial caraway fruit yield depends on the size of leaves rosette and root thickness reached in the first year of its vegetation (Seidler-Lozykowska et al., 2010).

As the seed is the only part of the plant used, the quality of the biennial form is considered to be superior to the annual one, according to size, aroma, color etc. (Bouwmeester et al., 1993), but lack of cultivation of these form of caraway is that in the first year does not provide benefits but requires investment.

In the moderate climate of Central Europe beside pure crop cultivation methods, and mixed cultivation methods is used (Nemeth, 1998). Mixed cultivation with a cover crop is usually preferred by the owners of relatively smaller farms located under favorable soil and climatic conditions. Those growers are orientated towards intensive usage of arable land. Cover crops are cultivated together with caraway and yield in the first year, being mainly represented by garden poppy, pea, coriander, spring oilseed rape, spring barely, chamomile, flax and spinach.

Weed control is a very important factor mainly during the early developing stages before plants cover the field, at early spring. During this period the ground is bare and winter weeds can grow very easily. From early spring the caraway plants cover the ground and normally (when there are enough plants per unit area) no addition of annual weeds can be observed. The growing season of biennial caraway is much longer, therefore a wide range of weed control is needed for a longer time. Most of the weed controls found suitable for the establishment phase of biennial caraway.

At difference of biannual forms, annual caraway forms a less rosette of leaves (usually four to five leaves), and quickly began developing umbel. The vegetation period of annual caraway usually lasts 140-160 days. Shorter growing period significantly reduced investment in growing practices, especially the competition with the weeds. For this reason, the aim of our study was to investigate the possibility of growing annual caraway in Serbia.

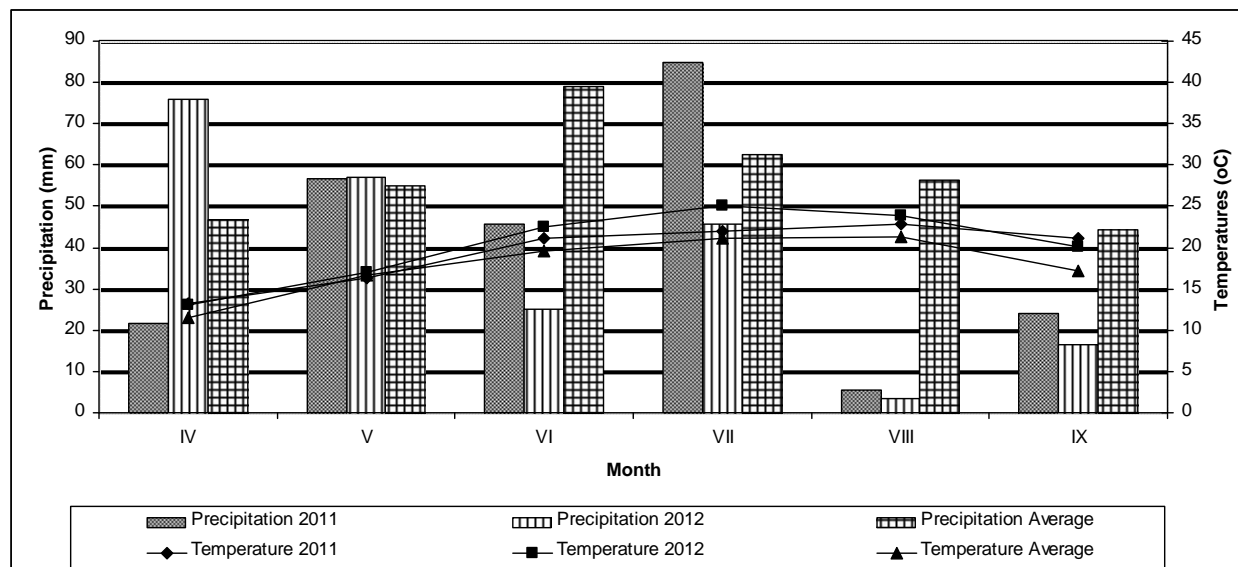
### **Material and method**

The field experiment was carried out during 2011 and 2012 years, on tree localities in Serbia, Vojvodina Province: (1) Mosorin (latitude 45°18'5" N; longitude 20°09'32" E; altitude 111 m), (2) Ostojicevo (latitude 45°53'16" N; longitude 20°09'31" E; altitude 88 m) and (3) Veliki Radinci (latitude 45°02'26" E; longitude 19°40'15" E; altitude 111 m).

Caraway seed (local ecotypes) obtained from medicinal plant farm from Kulpin were used in this study. Caraway is grown only from direct sowing, which is performed in rows at 35cm spacing during April. After germination it was performed thinned for obtaining 200 plants on meter square.

Seedlings emerge usually after 2 weeks and, like in other plants from *Apiaceae* family, their initial growth is slow. At that time the young plants are particularly sensitive to shading, and also to insufficient aeration and moisture of the soil. According this reasons it was need 2-3 hoeing. Harvest was performed in full ripening stage by hand. In the first experimental year, harvest was in September, and in second experimental year, one month earlier (i.e. during August).

Data of meteorological conditions were obtained from nearest meteorological station for each experimental field (<30 km), and average value of temperatures and precipitations for all tree localities, for both investigated years, as well as long time average of these parameters, is shown on Figure 1.



**Figure 1. Average monthly values of precipitations and temperatures for vegetation period (IV-X) in both experimental years and long time average (Source Republic Hydrometeorological service of Serbia)**

Soil samples were taken from 0-30 cm, and analyzed in Soil tested laboratory of Agricultural Extension Service, Sremska Mitrovica, and shown in Table 1. For determination pH soil was used potentiometric method, for  $\text{CaCO}_3$  molar volume of carbon dioxide, humus content was determine by Turin method, total nitrogen by Kjeldahl method, available phosphorous and potassium with Al-method, Egner-Riehem.

**Table 1. Agrochemical analysis of soil**

	pH (KCl)	$\text{CaCO}_3$ (%)	Humus (%)	Total Nitrogen (%)	$\text{AlP}_2\text{O}_5$ (mg/100 g)	$\text{AlK}_2\text{O}$ (mg/100 g)
Location 1	7.3	8.4	2.7	0.18	81.6	75.1
Location 2	7.3	8.8	2.2	0.14	17.6	30.3
Location 3	7.1	2.0	2.5	0.16	22.4	21.7

Data were subject to statistical analysis using the program package STATISTICA (Statsoft, 1998) and expressed as mean, variance ( $s^2$ ) and coefficient of variation (CV). The mean values were compared by using the one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. The differences between individual means were deemed to be significant at  $p < 0.05$ .

### Results and discussion

From table 2, it can be concluded that on the yield of annual caraway weather conditions during year have a statistically significant influence. In a favorable year (2011) has been achieved the average yield of  $850 \text{ kg ha}^{-1}$ , while in the dry year yield was reduced ( $193 \text{ kg ha}^{-1}$ ).

In a year that was favorable for the formation yields, it varied from  $460,16$  to  $1208,67 \text{ kg ha}^{-1}$ . According to the literature, yield of annual caraway varies from  $900 \text{ kg ha}^{-1}$  (Chevalho and Fonseca, 2006) up to  $1250 \text{ kg ha}^{-1}$  (Bailer et al., 2001). During a drought in 2012, the yield of caraway was very low ( $72,55$  to  $310,95 \text{ kg ha}^{-1}$ ). In our country has already recorded a very

low yield of caraway grown in Stara Pazova (Drazic et al., 1998), which was  $124 \text{ kg ha}^{-1}$ , and it has varied a lot ( $\text{CV}=53$ ).

Table 2: Yield of caraway fruits ( $\text{kg ha}^{-1}$ ) during two investigated years

	Yield	Variance	CV
2011	849,5 <sup>a</sup>	41961,27	24,11
2012	192,8 <sup>b</sup>	9023,40	49,28
Average	521,2	-	-

Mean values in a column with different letters (a, b) are significantly different at  $P < 0,05$  (Duncan's test).

It may be noted that the site significantly affects on formation yields, which is particularly evident in the year with unfavorable weather conditions as it was in 2012, with the coefficient of variation was 49,28%. The great variation of yields in the second year, most likely is the result of the uneven distribution of rainfall that had downpour character. That the yield of caraway a lot depends on the influence site, i.e. that the height and yield stability depend on the ability of the population to react to environmental factors indicated Drazic (1992).

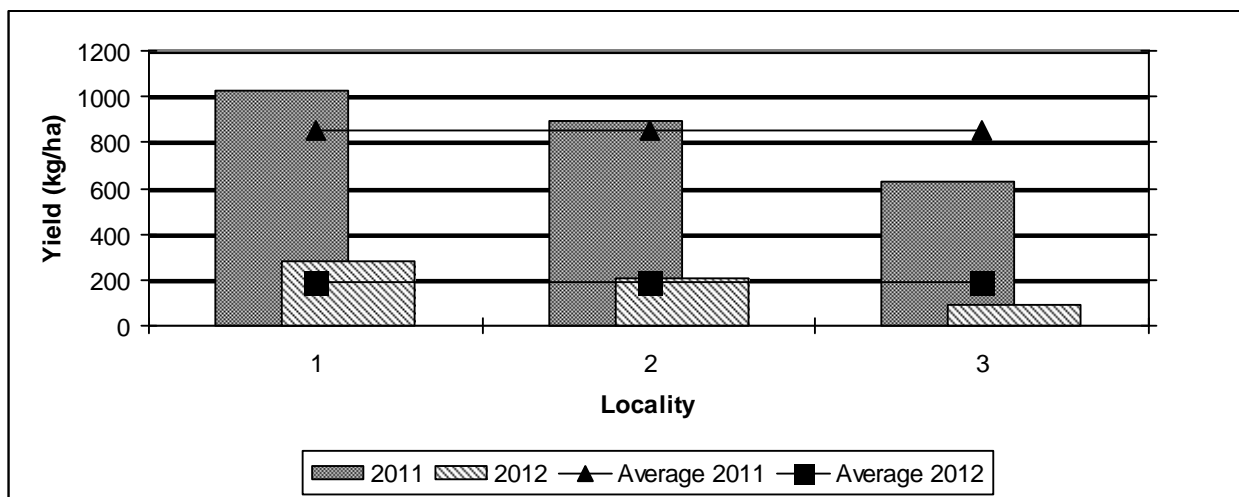


Figure 2. Yield of caraway fruits ( $\text{kg ha}^{-1}$ ) during two investigated years depending on locality

As it can be seen from figure 2, the highest yield in both investigated year was achieved on locality 1 ( $1027,7 \text{ kg ha}^{-1}$  in 2011, and  $279,8 \text{ kg ha}^{-1}$  in 2012), where was the highest amount phosphorus in soil ( $81,6 \text{ mg}/100 \text{ g soil}$ ). Phosphorus plays an important role in photosynthesis, respiration, energy storage, cell division/enlargement and several other processes like seed formation, hastening maturity and also contributes to disease resistance in coriander (Ibadullah et al., 2011).

Seed yields of caraway vary considerably between years at the same site and in the same year at different sites with the same variety. It means that environmental conditions affected the yield or determining processes of seed production (Nemeth, 1998).

Due to poor rainfall for the last few years in Poland the moisture in the soils has decreased and that has affected growth and yielding of many crops, including caraway. Water deficit causes changes in almost all the cell processes, which affected plant growth and development (Seidler-Lozykowska et al., 2010).

Water deficit is considered to be a major environmental factor affecting agriculture productivity worldwide and causing considerable crop yield reduction (Laribi et al., 2011).

According to Nemeth (1998), in semiarid regions where the annual caraway grows, there are two critical stages during its growth, where irrigation is necessary: from germination to establishment and at seed formation. The first stage appears at spring and the other at summer usually during July and August. At those periods the amount and date of rainfall change from year to year, so it is necessary artificial irrigation.

Seed yield seems to be determined mainly by the conditions during flowering Bouwmeester et al., (1995). Undoubtedly, weather conditions, i.e. rain, humidity and wind also affect pollination efficiency (Nemeth, 1998).

### Conclusion

The yield of annual caraway mostly depended on weather condition during year. In semiarid regions where the annual caraway grows, amount and date of rainfall change from year to year, and from site to site. From that case, it is impossible growing annual caraway in dry years in field condition without artificial irrigation.

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## THE EFFECT OF CYTOPLASMIC MALE STERILITY ON YIELD STABILITY OF MAIZE INBRED LINES

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### Abstract

Nowadays the production of hybrid maize seed is increasingly based on using cytoplasmic male sterility (CMS) in order to reduce costs of detasseling. Since inbred lines are unstable due to their performance, it is very important to investigate the influence of the specific type of sterility on yield stability. With the aim to analyse the effect of CMS on yield stability, seven maize inbred lines, developed at Maize Research Institute “Zemun Polje” were examined in this study. Each of these seven inbreds was investigated in five variants: with normal cytoplasm (N), with C type sterile cytoplasm (CMS-C), the fertile counterpart C (RfC), with S type sterile cytoplasm (CMS-S) and the fertile counterpart S (RfS). The yield was analysed using the method of Eberhart and Russell on the basis of the coefficient of linear regression. The inbred line ZPL-1 was the best ranked within inbreds with N, CMS-S and RfS type of germplasm. Therefore, this inbred line was most stable within the stated genotypes. On the other hand, values closest to one were detected in the inbred line ZPL-6 within the inbreds with CMS-C and RfC germplasm, so this inbred was the most stable for those two types of cytoplasm.

**Key words:** maize, yield, cytoplasmic male sterility, inbred lines

### Introduction

In many important field crops the CMS trait is used exclusively to produce commercial hybrid seeds. Using CMS to prevent self-fertilisation of the seed parent plant does away with the costly and labour-intensive manual or mechanical emasculation.

In a large number of investigations contradictory results have been reported with respect to differences in grain yields of CMS and normal fertile maize inbred lines and hybrids. Positive effects of CMS on grain yields were reported by Sanford (1965), Stamp (2000), Weingartner (2002) and Kaeser (2003). The absence of effect or inconsistent effects of CMS on grain yields were reported by Duvick (1958) and Everett (1960). A negative effect of CMS on grain yields was found for CMS hybrids with CMS-T cytoplasm (Stringfield, 1958), compared to the fertile counterpart, and by Noble and Russell (1963), who tested male-sterile and normal male-fertile cytoplasm.

If CMS increases the yield of inbred lines and hybrids, the generally accepted physiological explanation is the lower consumption of energy and nutrients in male-sterile tassels than in normal fertile tassels. This is advantageous for the female component of a male-sterile plant, because these stocks can be used for the development of the ear and grain filling (Kaeser et al., 2003).

Adaptability and stability of a genotype is expressed through the genotype x environment interaction. This interaction is a source of variation that includes a genotype and environmental effects. Individual consideration of these two factors does not give a real image of either a genotype or environments. Reliable results can only be obtained if the

interrelationship of these two sources of variation is included. The occurrence of intensive genotypes also increases susceptibility of genotypes to environmental conditions (Dimitrijević and Petrović, 2000).

Several models for testing adaptability and stability of yield have been developed during the last 70 years (Finlay and Wilkinson, 1963, Lin and Binns, 1988). The most widely used method for the analysis of stability was coined by Eberhart and Russell (1966). This method is based on the regression coefficient  $b_i$  and standard deviation from regression  $Sd_i^2$ .

The yields of maize inbred lines with normal cytoplasm as well as their CMS and Rf variants were observed in the present study. The stability parameter  $b_i$  was used in studying yield stability of inbreds.

### Materials and Methods

The following seven maize inbred lines of different origin and maturity groups (FAO 300-500) were used in this study: ZPL-1, ZPL-2, ZPL-3, ZPL-4, ZPL-5, ZPL-6 and ZPL-7.

Cytoplasmic male sterile and restorer versions of inbreds were developed by conversion of inbreds with normal cytoplasm at the Maize Research Institute, Zemun Polje.

Trials were set up according to the randomised split-plot design in three locations (Zemun Polje, Školsko dobro - a location within trial fields of the Maize Research Institute, Zemun Polje and Srbobran) in 2010 and 2011. All trials were set up under conditions of dry land farming. Sowing was always done on the optimum dates with the application of common cropping practices.

The trial encompassed three replications in five sets (blocks). Each block represented one of the type of observed inbreds:

I block – N (normal) cytoplasm, i.e. original inbred lines

II block – CMS-C inbreds

III block – RfC inbreds

IV block – CMS-S inbreds

V block – RfS inbreds .

Plots within a replication were composed of 4 rows, and each row had 12 hills at the distance of 40 cm. Sowing was done by hand with four plants per hill. The inter-row distance was 70 cm. The elementary plot size was 7.28 m<sup>2</sup>, while sowing density amounted to 71,429 plants ha<sup>-1</sup>. Thinning to two plants per hill was done in the 5-leaf stage. Plants from 10 inner hills were used for the analysis of grain yields (in order to avoid a border hill effect).

The grain yield was presented in t ha<sup>-1</sup> at 14% moisture.

The parameters for grain stability were estimated using method developed by Eberhart and Russell (1966), as indicators of seed production reliability on fertile and sterile basis under various environmental conditions.

### Results and Discussion

The analysis of stability parameters was done on the basis of grain yields of all seven inbreds within each block (inbred type). The regression coefficient ( $b_i$ ) showed the response of a genotype to the environment. When  $b_i=1$  average adaptability and stability of an observed genotype are uniform under both environmental conditions, favourable and unfavourable; when  $b_i>1$  a genotype is averagely stable only under favourable environmental conditions; and when  $b_i<1$  a genotype performance is better under poorer environmental conditions.

The values of average yields and stability parameters for inbreds with normal cytoplasm are shown in the Table 1. The highest average yields of 6.5727 t ha<sup>-1</sup> and 5.3533 t ha<sup>-1</sup> were recorded in the inbreds ZPL7 and ZPL2, respectively. The lowest yield of 3.7267 t ha<sup>-1</sup> was achieved by the inbred ZPL4. According to the regression coefficient the inbred ZPL1 was



the most stable, while the inbred ZPL7 with the lowest yield also had the poorest value of this coefficient. Performance of inbreds ZPL6 and ZPL7 was better under favourable growing conditions, while inbreds ZPL2 and ZPL5 were more productive under somewhat unfavourable conditions.

Table 1. Yields and stability parameters of inbreds with N type of germplasm

Inbred	Yield (t ha <sup>-1</sup> )	Rank	b <sub>i</sub>	Rank
ZPL1	4.498	5	1.0451	1
ZPL2	5.3533	2	0.8241	4
ZPL3	4.772	4	1.1312	3
ZPL4	3.7267	7	0.6397	7
ZPL5	4.2333	6	0.8978	2
ZPL6	4.9907	3	1.2610	6
ZPL7	6.5727	1	1.2122	5

Results obtained on average yields and stability parameters of inbreds encompassing C cytoplasm are presented in Table 2. The highest average yields of 6.5327 t ha<sup>-1</sup> and 5.91 t ha<sup>-1</sup> were recorded in inbreds ZPL7 and ZPL2, respectively, the same as in inbreds with N cytoplasm. On the other hand, the lowest yield (3.7267 t ha<sup>-1</sup>) was detected in the inbred ZPL4. Based on stability parameters, the most stable inbred according to the values of the regression coefficient was inbred ZPL6, while the most yielding inbred was at the same time the least stable (ZPL7). The inbreds ZL1, ZPL4 and ZPL5 performed better under less favourable growing conditions. The inbred ZPL2 expressed greater adaptability under favourable growing conditions.

Table 2. Yields and stability parameters of inbreds with C type of germplasm

Inbred	Yield (t ha <sup>-1</sup> )	Rank	b <sub>i</sub>	Rank
ZPL1	4.826	6	0.7838	4
ZPL2	5.91	2	1.3003	5
ZPL3	4.8407	5	1.0714	2
ZPL4	4.2633	7	0.8265	3
ZPL5	5.2027	4	0.6826	6
ZPL6	5.5453	3	0.9496	1
ZPL7	6.5327	1	1.3759	7

As for the inbreds with RfC type of germplasm (Table 3) the highest yield of 6.0883 t ha<sup>-1</sup> was recorded in the inbred ZPL2, while the inbred ZPL7 ranked second with the average yield of 5.4107 t ha<sup>-1</sup>. Based on the regression coefficient, the inbred ZPL6 was the most stable. According to this parameter the lowest stability was recorded in the inbred ZPL7. Under favourable growing conditions, better results were recorded in the inbreds ZPL1 and ZPL3, while the inbred ZPL5 was more adaptable to poorer conditions.

Table 3. Yields and stability parameters of inbreds with RfC type of germplasm

Inbred	Yield (t ha <sup>-1</sup> )	Rank	b <sub>i</sub>	Rank
ZPL1	4.4540	5	1.6714	6
ZPL2	6.0833	1	0.0102	7
ZPL3	4.7087	4	1.5396	5
ZPL4	3.9260	7	1.0223	2
ZPL5	4.2080	6	0.6262	4
ZPL6	5.1593	3	1.0131	1
ZPL7	5.4107	2	1.1070	3

The highest yield (6.5373 t ha<sup>-1</sup>) of inbreds with S type of germplasm was recorded in the inbred ZPL7 (Table 4). The inbred ZPL2 with the average yield of 5.89 t ha<sup>-1</sup> ranked second. The lowest yield was again recorded in the inbred ZPL4. The inbred ZPL1 had the regression coefficient closest to unit and as such was the most stable, although it ranked next to the last by its yield. Inbreds ZPL2 and ZPL3 responded better to favourable growing conditions, while inbreds ZPL4, ZPL5, ZPL6 and ZPL7 responded better to unfavourable environmental conditions.

Table 4. Yields and stability parameters of inbreds with S type of germplasm

Inbred	Yield (t ha <sup>-1</sup> )	Rank	b <sub>i</sub>	Rank
ZPL1	4.5127	6	1.0161	1
ZPL2	5.8900	2	1.5206	7
ZPL3	5.0793	4	1.2085	4
ZPL4	4.2940	7	0.8620	3
ZPL5	4.6600	5	0.7668	5
ZPL6	5.4027	3	0.7605	6
ZPL7	6.5373	1	0.8681	2

The inbred ZPL7, most yielding in previous studies, was also the most yielding inbred in the present study regarding genotypes with RfS germplasm (5.7587 t ha<sup>-1</sup>, Table 5). Moreover, the inbred ZPL4 was the lowest yielding inbred in both studies, previous and present. The inbred ZPL1 was the most stable one. Inbreds ZPL3, ZPL6 and ZPL7 had values of b<sub>i</sub> greater than one and therefore their performance was better under favourable growing conditions. Inbreds ZPL2, ZPL4 and ZPL5 were more adaptable to unfavourable environmental conditions.

Table 5. Yields and stability parameters of inbreds with RfS type of germplasm

Inbred	Yield (t ha <sup>-1</sup> )	Rank	bi	Rank
ZPL1	4.3930	4	0.8641	1
ZPL2	4.2667	6	0.8264	2
ZPL3	4.9253	2	1.3161	4
ZPL4	4.0847	7	0.6613	5
ZPL5	4.3060	5	0.5495	7
ZPL6	4.8960	3	1.2079	3
ZPL7	5.7587	1	1.5938	6

According to data obtained by the analysis of grain yields conducted by the method of Eberhart and Russell it can be concluded that out of seven observed inbreds the inbred ZPL7 was the most yielding for all types (blocks) except for the RfC type within where it was ranked second. Considering the grain yield, this inbred is also the most suitable for the seed production especially if it is used as a female component. On the other hand, the inbred ZPL4 had the lowest yields in all types (blocks) and therefore it can be recommended that this inbred is used as a male component. The remaining inbreds have a relatively good yield, and therefore they can be used in the maize hybrid seed production either as female or male components. Based on the regression coefficients, the inbred ZPL1 was ranked first within inbreds with N, S and RfS type of germplasm, and as such it can be considered the most stable inbred. The inbred ZPL6 had values closest to one within the inbreds of C- and RfC-type, while the inbred ZPL1 ranked fourth and sixth within C-type and RfC-type inbreds, respectively. The conclusion on which inbred has the poorest values of this parameter over different types (blocks) of inbreds cannot be drawn.

Studies by Kaeser et al. (2003) and Kaul (1988) indicate that, under high-yielding conditions CMS-S can be inferior to other CMS types with regard to the yield increase. This may be due to the fact that processes leading to pollen sterility are initiated by the CMS-S cytoplasm at a very late development stage of pollen production. The CMS-C cytoplasm interrupts pollen production at an earlier stage than does CMS-S cytoplasm, which can result in a more favourable translocation of nutrients to the ear primordia. In our research we have not observed that CMS-S inbreds are inferior comparing to inbreds with CMS-C cytoplasm. In our study, both CMS-S and CMS-C inbreds showed higher yields comparing to inbreds with normal cytoplasm and their fertile counterparts.

### Conclusions

The effect of CMS on yield stability in seven maize inbred lines developed at Maize Research Institute “Zemun Polje” was observed in the present study. Each of these seven inbreds were investigated in five variants: with normal cytoplasm (N), with C type sterile cytoplasm (CMS-C), the fertile counterpart C (RfC), with S type sterile cytoplasm (CMS-S) and the fertile counterpart S (RfS). As it had been expected, it was shown that inbreds with both C and S sterile cytoplasm had higher yields comparing to normal and Rf variants. We could say that all analysed inbreds had good yield, and therefore could be used in seed production. There was no difference shown between two examined types of sterility. Based

on the  $b_i$  coefficient of stability, the most stable inbred for N, CMS-S and RfS variants was ZPL1, while ZPL6 was the most stable inbred for CMS-C and RfC types of germplasm.

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## GRAIN YIELD AND YIELD STABILITY OF ZP MAIZE HYBRIDS IN DROUGHT CONDITIONS IN SERBIA

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### Abstract

Selection of maize hybrids in Maize Research Institute “Zemun Polje”(ZP) exists for over 60 years. In this study 12 ZP hybrids from different maturity groups from FAO 300-600 and two foreign hybrids as checks were tested. Macro experiments were set up at 18 locations throughout Serbia during 2012. The past 2012<sup>th</sup> year was extremely unfavorable for corn production. Besides significant lack of rainfall, grain yield was reduced and affected by the extreme heat during pollination and grain filling period of maize. The average yield on the 18 selected sites was 5.87 t/ha. The highest yield was obtained by ZP 505 (6.39 t/ha). Mid-early and mid-late maturing hybrids have achieved better results on average compared to a long growing season hybrids. The most stable is the new hybrid ZP 427, which showed equally well to both favorable and unfavorable growing conditions. Besides mentioned hybrid, high stability was obtained by hybrid ZP 505. ZP 434 was the most unstable hybrid, which showed significantly better adaptation to poorer growing conditions, as well as hybrids ZP 548 and ZP 555, which also showed unstable, but better adapted to favorable growing conditions. ZP 341 obtained the best performance in poor growing conditions. Based on the results of the tests, it is concluded that ZP hybrids had good performance in agroecologically unfavorable 2012<sup>th</sup> year. Mid-early and mid-late maturing hybrids (FAO 300-500) are recommendation for dry areas and years in Serbia, which was concluded by their both, grain yield and stability.

**Key words:** maize, grain yield, stability performance, drought

### Introduction

Maize is one of the leading crops in world's agriculture. When it comes to area sown, maize finds itself on third place after wheat and rice (Glamo lija, 2004), while in Serbia it occupies the first place on an average of 1.2 million hectares sown annually. Serbia has two production areas, Vojvodina (northern province of Serbia) and Central Serbia. The main difference is that the climate is drier and harsher in Central Serbia, ie. primarily reflected in lower rainfall during the vegetation period and higher altitude compared to Vojvodina.

Maize breeding program in Zemun Polje exists for over 60 years and all created hybrids can be divided into hybrids of the six cycles of selection (<http://www.mrizp.rs/en/research-l/breeding> ). Each next cycle produced hybrids for more intensive production conditions than the previous one. They were more modern than previous, had bigger yield potential and were capable of handling the needs of producers on one side and the problems of abiotic and biotic stresses on the other. Besides selection for high and stable yield, maize growing technology of also changed. It is believed that about 60% increase in grain yield is resulting from new hybrid creation, while the remaining 40% represents a result of new technological solutions and education of maize producers (Jockovi et al. 2010).

Climate change puts a difficult task before the maize breeders. The production of hybrids adapted to the conditions of temperature increase in Serbia and a decrease in precipitation represents new task of breeders. Increase in temperatures throughout Vojvodina and the reduction of rainfall in the same area is expected for future times (Lalic et al. 2011).

When testing new hybrid, it is necessary to do statistical analysis, with which conclusions can be given about the quality of newly developed hybrids. For each hybrid is essential that high yield is accompanied by a maximum yield stability. The stability of hybrids is best to be checked in adverse and favorable environmental conditions. The past 2012<sup>th</sup> years in Serbia was very dry with high temperatures and lack of rainfall through maize vegetation period. One way to check hybrids is to test them at greater number of locations. While testing the stability of hybrids, it is possible to happen that the highest yielding hybrids show a below-average stability (Babic, 2006). The high stability of genotypes is one of the most desirable features as one of the main preconditions for the expansion of the same hybrids for cultivation in large areas (Singh and Choudhary, 1977).

Maize Research Institute "Zemun Polje" has in its offer hybrids of all FAO maturity groups (FAO 100-800). Earlier, as the most cultivated hybrids were those of later maturity groups (FAO 600-700). Today, there is an increasing demand for hybrids FAO 300-500, because they are hybrids that spend their growing period in terms of somewhat better supply of soil moisture and lower air temperatures. The aim of this study was to examine the productivity of commercial maize hybrids yield variability, and to determine the stability of grain yield of hybrids in the dry 2012<sup>th</sup> year.

#### Materials and methods

In this study 12 ZP maize hybrids were investigated from different maturity groups of FAO 300-600 and two foreign hybrid checks. All hybrids are grown and commercial, and are widespread in the whole of Serbia. Besides our hybrids, two foreign checks were also used in order to get a better picture and clearer validation of our hybrids. Checks were representatives from maturity groups FAO 500 (ch 500) and FAO 600 (ch 600).

Macro trials were set up at 18 locations through out Serbia during the year 2012: Zemun Polje (ZP), Divoš (DI), Sremska Mitrovica (SM), Kuzmin (KU), Bečej (BE), Zmajevo (ZM), Deronje (DE), Botoš (BO), Izbište (IZ) representing Vojvodina province and Loznica (LO), Mavanski Prnjavor (MP), Šalinac (ŠA), Aleksandrovac (AL), Crnoklište (CR), Kurjaci (KU), Smederevo (SD), Svilajnac (SV) and Leskovac (LE) representing Central Serbia. Selected locations cover the major production areas in the country.

Plot size was 0.1 ha. Sowing and harvesting operations are carried out mechanically. Plant density per area depended on the maturity group, and was performed at three different densities: FAO 300-400: 70.000 plants per hectare, FAO 500-600: 65.000 plants per hectare and 600 FAO: 60.000 plants per hectare. The yield was calculated in tonnes per hectare (t/ha) at grain moisture of 14%.

For the purposes of this paper average yields were calculated separately for Central Serbia and Vojvodina, for the sake of determination of difference in yield production areas, on the other hand, stability parameters were performed using Eberhart and Russell (1966) for all 18 locations together.

Eberhart and Russell method is represented by the following equation:

$$Y_{ij} = m + b_i I_j + d_{ij}$$

$Y_{ij}$  - the average yield of the  $i^{\text{th}}$  genotype in the  $j^{\text{th}}$  location

$m$  - the average yield of the  $i^{\text{th}}$  genotype at all locations

$b_i$  – standardised regression coefficient of the environment and genotype, genotype relationship to the environment

$I_j$  - index of the environment as the average of all genotypes in the  $j^{\text{th}}$  location, reduced from the total average

$d_{ij}$ -deviation from regression of the  $i^{\text{th}}$  genotype in the  $j^{\text{th}}$  site

Standardized regression coefficient ( $b_i$ ) shows the response of genotypes to environment. When  $b_i=1$ , then the average adaptability and stability of the tested genotypes was considered uniform under the influence of favorable and poor environment conditions; when  $b_i > 1$ , genotype was considered stable and good only in favorable environment conditions, and when the  $b_i < 1$ , genotype was considered to perform better than the average in less favorable growing conditions and environments. All calculations were done in Excel programme.

### Results and discussion

The results of grain yield trials on all 18 locations and stability parameters are shown in Table 3, and grain yields of hybrids tested on locations of production areas of Vojvodina and central Serbia are shown in Tables 1 and 2.

Achieved grain yield in Vojvodina province and Central Serbia show difference between yields of hybrids on different locations of the same area of production, and between two areas themselves (Tables 1 and 2). When it comes production areas average grain yield of all hybrids was 6.11 t/ha and 5.63 in Vojvodina and Central Serbia respectively, with a difference in grain yield between Vojvodina and Central Serbia nearly 0.5 t/ha (0.49 t/ha).

Grain yields of hybrids ranged from 5.36 t/ha (ZP 648) to 6.82 t/ha (ZP 505) in Vojvodina and 4.75 t/ha (ZP 684) to 5.96 t/ha (ZP 505) in Central Serbia. ZP 505 showed the best result in both production areas. Vojvodina province, on all locations has eight hybrids reaching yield above 6 t/ha, where none of tested hybrids managed to cross the line of 6 t/ha in Central Serbia.

**Table 1.** Grain yield of ZP hybrids on locations in Vojvodina province (t/ha)

Hybrid	ZP	DI	SM	KU	BE	ZM	DE	BO	IZ	Average
ZP 341	9.05	4.84	5.05	4.33	7.92	5.72	5.88	7.09	7.53	6.38
ZP 427	9.41	3.60	4.50	2.77	8.47	5.69	6.32	6.69	8.10	6.17
ZP 434	8.39	4.71	5.07	4.37	7.05	6.03	6.74	7.22	8.19	6.42
ZP 505	9.00	4.02	4.75	5.11	8.91	6.44	6.60	7.62	8.90	6.82
ZP 548	8.74	3.67	3.95	4.42	7.56	5.04	6.65	6.87	8.77	6.19
ZP 555	9.64	4.24	5.43	4.42	8.79	5.77	5.42	7.56	8.21	6.61
ZP 560	7.75	3.10	5.31	4.30	8.33	6.00	3.74	7.53	8.43	6.05
ZP 600	7.57	3.28	5.13	4.27	7.34	5.66	3.59	7.43	8.79	5.89
ZP 606	8.17	3.66	5.37	3.77	7.58	5.31	4.23	7.03	8.51	5.96
ZP 648	8.06	2.52	5.00	2.94	7.52	3.83	4.09	5.91	8.32	5.36
ZP 666	8.45	4.56	5.86	3.97	8.58	5.27	4.48	6.77	8.69	6.29
ZP 684	6.84	3.81	5.21	3.32	7.19	4.43	4.95	6.60	8.28	5.63
ch 500	6.41	3.76	6.74	4.30	7.70	5.37	2.65	7.19	7.72	5.76
ch 600	6.14	4.02	6.16	2.87	9.01	5.16	4.08	7.58	8.29	5.92
Average	8.12	3.84	5.25	3.94	8.00	5.41	4.96	7.08	8.33	6.11

When it comes to locations alone, average grain yield ranged from 3.84 t/ha (location Divoš) to 8.33 t/ha (location Izbište) with interval of yield variation of 4.49 t/ha in Vojvodina, and 3.07 t/ha (location Crnoklište) to 8.60 t/ha with interval of yield variation of 5.53 t/ha in Central Serbia.

**Table 2.** Grain yield of ZP hybrids on locations in Central Serbia (t/ha)

Hybrid	LO	MP	ŠA	AL	CR	KU	SD	SV	LE	Average
ZP 341	4.85	7.57	9.12	5.89	3.48	6.11	4.42	6.87	4.35	5.85
ZP 427	4.87	7.17	7.88	6.00	2.96	4.65	4.75	5.55	5.67	5.51
ZP 434	4.81	7.23	8.03	6.73	3.11	5.70	4.13	6.77	4.60	5.68
ZP 505	4.77	7.93	8.89	7.27	3.59	4.71	5.02	6.32	5.17	5.96
ZP 548	5.32	7.43	9.82	7.18	2.47	3.74	3.37	7.26	5.71	5.81
ZP 555	4.52	8.01	9.53	7.65	3.83	3.66	3.89	7.70	4.53	5.92
ZP 560	4.06	7.59	9.01	7.72	2.72	3.05	5.24	6.34	5.78	5.72
ZP 600	3.72	7.77	8.42	7.32	3.34	5.47	5.52	6.22	5.74	5.95
ZP 606	3.56	7.41	8.32	5.09	3.61	4.87	5.30	6.10	6.06	5.59
ZP 648	3.09	7.54	8.36	5.14	2.52	5.16	3.63	5.48	4.72	5.07
ZP 666	3.71	6.75	8.42	5.27	3.95	5.12	5.98	7.42	6.34	5.88
ZP 684	3.12	6.82	6.88	5.33	2.10	3.71	3.82	6.30	4.63	4.75
ch 500	3.99	8.07	8.45	5.42	3.10	4.03	5.53	6.57	5.30	5.61
ch 600	4.22	7.80	9.28	5.75	2.23	4.70	4.87	6.19	4.63	5.52
Average	4.19	7.51	8.60	6.27	3.07	4.62	4.68	6.51	5.23	5.63

Looking at the summing results of grain yields in the Table 3, hybrids of early medium maturity group FAO 300-400 fully managed to cope with hybrids of latter maturity hybrids in 2012<sup>th</sup> year, who usually achieve better yields. Hybrids ZP 341 and ZP 434 reaching 3<sup>th</sup> and 6<sup>th</sup> place in rank according to obtained grain yields of 14 hybrids. This could be understood by their earlier polination and start of grain filling period, while soil humidity and air temperatures are still good. A situation where the highest yielding hybrid does not need to be necessary the most stable one is repeated here with the hibrid ZP 505. ZP 427 with 5.83 t/ha on all 18 locations, ranking 9<sup>th</sup> was the most stable one with  $b_i=1.00$ . ZP 505 on the other hand achieved the highest grain yield, but ranked at 2<sup>nd</sup> place in a rank according to  $b_i$  with  $b_i=1.01$ , showing excellent result in dry 2012. year. The lowest yielding hybrid in Serbia in 2012<sup>th</sup> year was ZP 684, but showed good stability with a 3<sup>rd</sup> place in rank according to  $b_i$ .



**Table 3.** Grain yield of commercial ZP hybrids on all 18 locations in production trials in 2012. year

Hybrid	Grain Yield (t/ha)	Rank according to grain yield	Moisture content at the time of harvest (%)	$b_i$	Rank according to $b_i$
ZP 341	6.12	3	13.0	0.89	6
ZP 427	5.83	9	12.9	1.00	1
ZP 434	6.05	6	13.6	0.82	9
ZP 505	6.39	1	15.2	1.01	2
ZP 548	6.06	5	15.5	1.15	8
ZP 555	6.27	2	15.2	1.15	8
ZP 560	5.88	8	15.5	1.12	7
ZP 600	5.92	7	15.7	1.09	4
ZP 606	5.78	10	15.8	0.94	3
ZP 648	5.21	13	16.2	1.12	7
ZP 666	6.09	4	15.6	0.90	5
ZP 684	5.19	14	16.7	0.94	3
ch 500	5.68	12	14.2	0.90	5
ch 600	5.72	11	14.7	1.09	4
Average	5.78	/	15.0	1	/

Hybrid with the lowest stability was ZP 434 with  $b_i$  far lower from 1, ie. 0.82, proving his superiority in poorer production conditions but reaching only number 6 in rank according to grain yield. ZP 666 is latter maturity hybrid but managed to gain high yield, ranking 4<sup>th</sup> place, but also had  $b_i=0.90$  showing his better adaptability for poorer growing conditions in comparing to other FAO 500-600 maturing hybrids. The hybrids with the highest  $b_i=1.15$  were ZP 548 and ZP 555, where ZP 555 was 2<sup>nd</sup> highest yielding hybrid and ZP 560 with  $b_i=1.12$ , leading to conclusion that these hybrids are more adaptable for better growing conditions, where they obtain very high results.

Two foreign checks ch 500 and ch 600 they showed 12<sup>th</sup> and 11<sup>th</sup> in ranks according to achieved grain yield and 4<sup>th</sup> and 5<sup>th</sup> place in rank according to  $b_i$ . One advantage that foreign checks showed was lower moisture content at the time of harvest with 14.2 % and 14.7 % respectively, putting them in category of hybrids for harvesting by grain shelling.

## Conclusion

ZP hybrids showed very good performance in 2012<sup>th</sup> year considering agroecological conditions in the same year. Yields in Vojvodina and Central Serbia showed that Vojvodina has little bit more favorable conditions, with a difference of 0.49 t/ha. Furthermore, Vojvodina also showed smaller variation in grain yield, concluding more equable growing conditions than Central Serbia who had highest yield obtained in Šalinac, but also lowest yield obtained in Crnoklište of both production areas. Also, looking at the grain yields of hybrids it self, eight of them managed to score yield over 6 t/ha in Vojvodina compared to none reaching such result in Central Serbia.

Hybrids of latter maturity groups (FAO 500-600) mostly obtained  $b_i$  higher than 1, showing better adaptability for more favorable growing conditions. Such representatives are primarily ZP 548, ZP 555 and ZP 560. ZP 505 showed the best results considering both grain yield and stability, and is recommended for production in dry areas in Serbia. Hybrids of FAO 300-400 maturity group never crossed  $b_i$  of 1, showing better adaptability to poorer growing conditions. Hybrid ZP 434 and ZP 341 with their achieved results are definitely recommended for dry and unfavorable locations in Serbia. This statement is in accordance with Pavlov et al. (2011), who also mentioned redistribution of maize hybrids of early and early medium maturity group for poorer and latter maturity maize hybrids for better agroecological growing conditions. The exception is hybrid ZP 666, being a latter maturity hybrid, but showed very good result in grain yield. Being the only hybrid with  $b_i=0.90$  and belonging to FAO 600 maturity group, and still ranking 4<sup>th</sup> in grain yield shows his great drought and high temperature tolerance, and is recommendation for dry areas with poorer growing conditions.

When it comes to comparison of ZP hybrids and two foreign checks, it can be concluded that maize breeding programme in Maize research Institute „Zemun Polje“ completely follows foreign maize breeding programme in grain yield and stability, but lacks grain dry down rate, where two FAO 500-600 foreign checks had lower moisture content in harvesting period than ZP maize hybrids of the same maturity group, allowing them harvesting directly in grain by shelling, rather than harvesting in cobs.

## Acknowledgments

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## EFFECTS OF 1-METHYL-CYCLOPROPENE ON THE PHYSICO-CHEMICAL PROPERTIES OF CHERRY FRUIT DURING STORAGE

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### Abstract

This study examined the effects of 1-methylcyclopropene (1-MCP) on basic physico-chemical characteristics of sweet cherry fruits, 'Burlat' cultivar. The fruits were exposed to 1-methylcyclopropene at concentrations of 0.05, 0.1 and 0.5 ppm, for 20 hours at 5°C. After treatment, the fruits were stored in the cold room (3°C) with normal atmosphere for 15 days, followed by 5 days storage at room temperature (shelf life). The average fruit weight, fruit firmness and total soluble solids in the fruit juice were determined and compared to the average values of these parameters before treatment. The obtained results indicate that 1-methylcyclopropene has an impact on the studied parameters. Fruit weight loss was the lowest in samples treated with 0.05 ppm 1-MCP and the highest at 0.5 ppm 1-MCP. The average fruit firmness was the lowest in the fruits treated with 0.05 ppm 1-MCP, and the highest in the non-treated fruits. The average value of the soluble solids content in the fruit juice was the lowest in fruits treated with 0.05 ppm, and the highest at 0.1 ppm.

**Keywords:** cherry 'Burlat', 1-methylcyclopropene, fruit storage

### Introduction

One of the important factors that influence fruit preservation is gaseous phytohormone ethylene, which at low concentrations activates the process of senescence (aging) of fruit, ultimately leading to deterioration of stored products. Negative impact of ethylene in fruit storage environment can be reduced or eliminated by using different alkene cyclic derivatives such as 2,5-norbornadiene; 3,3-dimethylcyclopropene; diazocyclopentadiene and 1-methylcyclopropene (Sisler et al., 1990). These compounds have the ability to inhibit ethylene action by binding to the ethylene receptors faster than ethylene itself (Sisler & Serek, 1997, 1999). Only 1-methylcyclopropene is used for commercial purposes so far (commercial formulations SmartFresh<sup>TM</sup> and EthylBlock<sup>TM</sup>) in order to control ethylene level in the cold rooms where horticultural products are stored. Previous studies have shown that 1-methylcyclopropene has a significant impact on the biochemical processes in climacteric fruits during ripening and storage, while the impact of this compound on non-climacteric fruits is contradictory (Porat et al., 1999; Mullins et al., 2000; Tian et al., 2000; Jiang et al., 2001). Cherry fruits are non-climacteric fruits (Biale, 1960; Blanpied, 1972) characterized by a low level of endogenous ethylene production (Biale and Young, 1981). Influence of 1-methylcyclopropene on the physico-chemical characteristics of cherry is not well documented. Yiping et al. (2002) reported that treatment with 1-methylcyclopropene did not affect respiration rate, fruit skin color, stem color and fruit firmness of the Bing and Rainier cherry cultivars. On the other hand, Yang et al. (2011) reported that treatment of cherry with 1-methylcyclopropene (EthylBlock formulation) is an effective method for fruit quality preservation during storage. Obviously, there is a need for further evaluation of the effects of 1-methylcyclopropene on cherry fruit preservation. The aim of this study was to investigate

the influence of 1-methylcyclopropene on basic physico-chemical characteristics of cherry fruit during storage in the cold room with normal atmosphere.

### Materials and methods

Fruits of the 'Burlat' cultivar were harvested from the Jablanica orchard, Gradiska. Trees from which the fruit was harvested were previously grafted on Gisela 5 and they were in the sixth year of cultivation. After harvesting at the consumption maturity stage the fruits were pomologically analyzed, determining the average fruit weight, soluble solids content in the fruit juice and fruit firmness. Soluble solids content was determined using a digital Atago refractometer. Fruit firmness was determined using a digital dynamometer (piston width of 1.5 cm and vertical path of 2 mm). Fruit skin was not removed and the fruit resistance to piston pressure was measured, rather than piston penetration into the fruit flesh. After the initial analysis, the fruits were exposed to 1-MCP at three different concentrations: 0.05, 0.1, and 0.5 ppm. SmartFresh formulation with 0.14% 1-MCP was used in the following way: 80 mg/m<sup>3</sup> (for 0.05 ppm concentration), 160 mg/m<sup>3</sup> (for 0.1 ppm) and 800 mg/m<sup>3</sup> (for 0.5 ppm concentration). 1-MCP solution was prepared by dissolving adequate amount of 1-MCP in 2 ml of distilled water at 25°C. The fruits were kept in a sealed container with 1-MCP for 20 hours at 5°C. After treatment, the fruits were stored in a chamber with normal atmosphere at 3°C and relative humidity of 95% for 15 days, and then kept at room temperature for 5 days (shelf life). The average fruit weight, soluble solids content and fruit firmness were determined and compared to the average values prior to the treatment.

The loss in fruit weight is expressed as weight loss (spoilage, moisture loss) and it was measured after 15 days of storage and 5 days of shelf life. Fruit weight was measured on the composite sample in a container that contained 40 fruits. Fruit flesh firmness and soluble solids content in the juice 10<sup>th</sup> and 15<sup>th</sup> day of storage, and after 5<sup>th</sup> day of shelf life. For the statistical analysis of data IBM SPSS Statistics 20 software was used. An analysis of variance with two factors (4 × 3) was done. Due to observed significant effect or interaction further analysis was performed using LSD test. Average values and standard error of arithmetic mean for the measured characteristics are also given.

### Results and Discussion

Table 1. shows measured weight values of the composite samples of cherry prior to the treatment with 1-MCP and after the storage and shelf life, as well as fruit weight loss after the storage and shelf life, and the total weight loss during the research.

By examining data given in Table 1 we can see that the smallest total weight loss was observed in fruits treated with 0.05 ppm 1-MCP (28.40 g), and the highest loss was observed in fruits treated with 0.5 ppm 1-MCP (43.40 g). Based on tabular and graphical data (Graph 1), we can see that the ratio of fruit weight loss during storage and during the shelf life is different for examined concentrations. Higher weight loss was observed during the cold storage in compared to shelf life at fruits which were exposed to the concentrations of 0.1 and 0.5 ppm of 1-MCP as well as with untreated fruits. Contrary to this, the higher weight loss during the shelf life compared to the cold storage was observed at fruits exposed to concentration of 0.05 ppm of 1-MCP.

**Table 1:** Composite samples fruit wight loss (40 fruits in a container) during the storage and after shelf life for different 1-MCP concentrations

1-MCP	initial weight (g)	weight after 15 days of storage (g)	weight after shelf life (g)	weight loss during storage (g)	weight loss during shelf life (g)	total weight loss (g)
0.05 ppm	200.1	188.3	171.7	11.8	16.6	28.4
0.1 ppm	208.3	190.3	179.5	18.0	10.8	28.8
0.5 ppm	204.1	171.5	160.7	32.6	10.8	43.4
non-treated	216.5	198.6	185.6	17.9	13.0	30.9

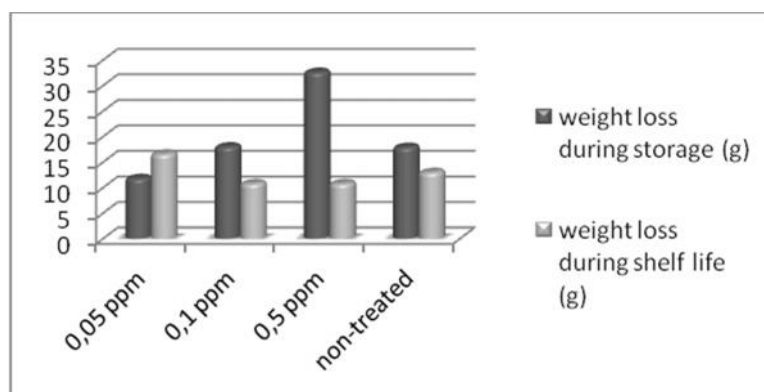
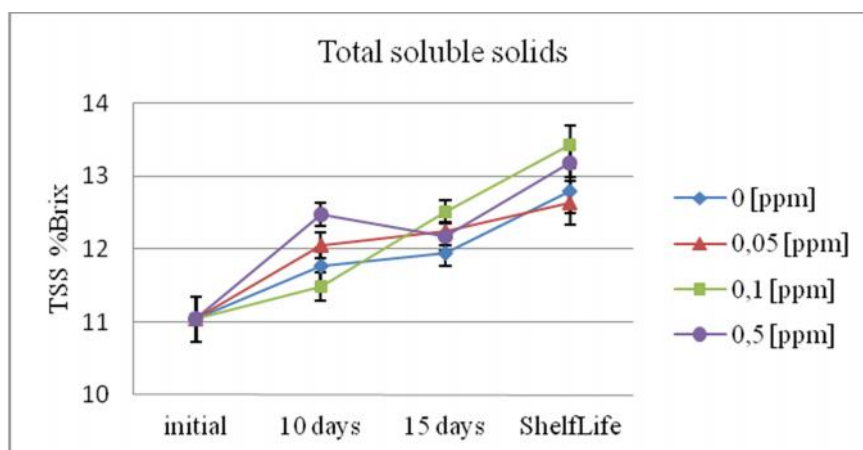

**Graph 1:** Graphical representation of weight loss during storage and during shelf life of fruits exposed to different concentrations of 1-MCP

Table 2. shows average values and standard error of the soluble solids content in fruit flesh juice of examined cherry cultivar for differenet 1-MCP concentratiois, before the treatment, after the cold storage and shelf life. Graph 2. shows graphical representation of examined paremeter.

**Table 2:** Average values and standard error of souluble solids content (Total soluble solids) (%Brix) in fruit flesh juice of examined cherry cultivar before, during and after the treatment at different concentrations of 1-MCP.

Storage period	1-MCP concentration [ppm]			
	0	0.05	0.1	0.5
	$\mu \pm Sx$	$\mu \pm Sx$	$\mu \pm Sx$	$\mu \pm Sx$
Initial	11.04 $\pm$ 0.31	11.04 $\pm$ 0.31	11.04 $\pm$ 0.31	11.04 $\pm$ 0.31
10 days after storage	11.77 $\pm$ 0.24	12.05 $\pm$ 0.18	11.48 $\pm$ 0.19	12.47 $\pm$ 0.16
15 days after storage	11.95 $\pm$ 0.19	12.24 $\pm$ 0.19	12.51 $\pm$ 0.16	12.17 $\pm$ 0.19
Shelf life	12.80 $\pm$ 0.31	12.64 $\pm$ 0.30	13.42 $\pm$ 0.28	13.18 $\pm$ 0.20



**Graph 2.** Graphical representation of the average soluble solids content values (%Brix) in fruit flesh juice of examined cherry before, during and after the treatment at different 1-MCP concentrations.

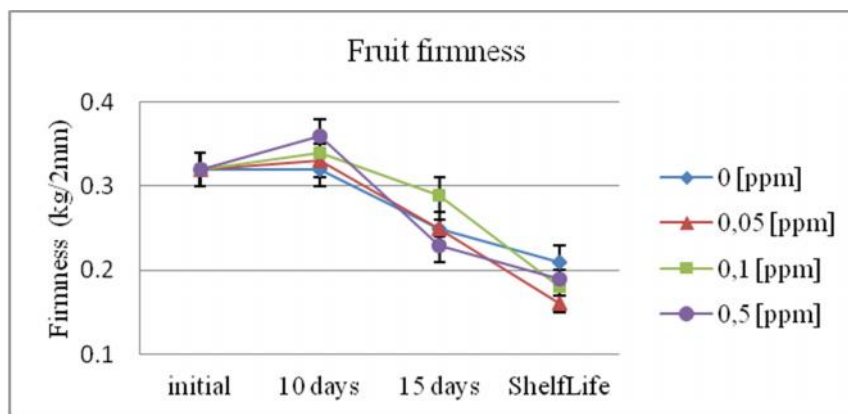
Based on data given in Table 2 and Graph 2, we can see that, as it would be expected, soluble solids content in fruit flesh juice increases with time. The smallest change for examined parameter was observed in cherry fruit which were treated with 0.05 ppm concentration of 1-MCP, and highest growth was observed in fruits treated with 0.1 ppm concentration of 1-MCP.

Table 3. shows average values and standard error for fruit flesh firmness in examined cherry cultivar at different concentrations of 1-MCP before the treatment, after the storage and shelflife, and Graph 3. gives graphical representation of examined parameter.

**Table 3.** Average values and standars error for fruit flesh firmness (kg/2mm) of examined cherry cultivar before, during and after the treatment at different concentrations of 1-MCP

Measuring moment	1-MCP concentration [ppm]			
	0	0.05	0.1	0.5
	$\mu \pm Sx$	$\mu \pm Sx$	$\mu \pm Sx$	$\mu \pm Sx$
Initial	$0.32 \pm 0.02$	$0.32 \pm 0.02$	$0.32 \pm 0.02$	$0.32 \pm 0.02$
10 days after storage	$0.31 \pm 0.02$	$0.33 \pm 0.02$	$0.34 \pm 0.02$	$0.36 \pm 0.02$
15 days after storage	$0.25 \pm 0.01$	$0.25 \pm 0.02$	$0.29 \pm 0.02$	$0.23 \pm 0.02$
Shelf life	$0.21 \pm 0.02$	$0.16 \pm 0.01$	$0.18 \pm 0.01$	$0.19 \pm 0.01$

Based on data from Table 3 and Graph 3, we can se that the smallest loss in fruit firmness was observed with untreated fruits, and the highest loss was observed at fruits treated with 1-MCP of 0.05 ppm concentration.



**Graph 3.** Graphical representation of average values of fruit flesh firmness (kg/2mm) of examined cherry cultivar before, druing and after the treatment at different 1-MCP concentrations.

**Table 4.** Analysis of variance for totale soluble solids and fruit firmness, F values and p values ( $p < 0,05^*$ ,  $p < 0,01^{**}$ )

Fruit characteristics	Factors		
	Storage period	1-MCP concentration	Interaction Time $\times$ MCP
TSS	25.45**	2.26	2.59*
Firmness	80.34**	0.97	1.65

The analysis of variance for soluble solids indicates statistically significant interaction between storage time and concentration of 1-MCP. By performing further breakdown of the factors and LSD test ( $p < 0.05$ ) it is indicated that fruits treated with 0.05 ppm showed no statistically significant difference in average values of total soluble solids between measurements in the 10<sup>th</sup>, 15<sup>th</sup> day and shelf life period. On the other hand, the fruits treated with 0.1 ppm expressed a statistically significant increase in the average values of total soluble solids in aforementioned measuring periods. At the 10<sup>th</sup> day of storage smallest total soluble solids values had fruits treated with 0.1 ppm 1-MCP, which for the 10<sup>th</sup> day is statistically significantly different only from fruits treated with 0.5 ppm. In the 15<sup>th</sup> day of storage no statistically significant differences have been observed between the fruits treated with different 1-MCP concentrations. In the shelf life period fruits treated with 0.1 ppm have the highest average total soluble solids content compared to other fruits treated with other concentrations of 1-MCP, where significant difference between these fruits and fruits treated with 0.05 ppm and 0 ppm of 1 – MCP was observed. During the shelf life the lowest average total soluble solids content have fruits treated with 0.05 ppm where significant difference is observed only in comparison with fruits treated with 0.1 ppm. There was no significant difference between the periods from 10<sup>th</sup> to 15<sup>th</sup> day for all 1-MCP treatments except for the treatment with 0.1 ppm where there was a statistically significant increase. By performing the analysis of variance of the average value of the fruit firmness a significant impact of the storage duration was observed. There was no statistically significant effect of different concentrations of 1-MCP, or interactions between the studied factors. By further analysis of the factors of storage time with LSD test ( $p < 0.05$ ) it was observed that there was statistically significant difference between the average values of fruit firmness measured on the 10<sup>th</sup>, 15<sup>th</sup> day and shelf life period.



### Conclusions

The research results of the effect of 1-MCP on basic physico-chemical characteristics of the cherry fruit, 'Burlat' cultivar, show that this agent has a certain influence on the fruit post-harvest physico-chemical characteristics during the storage process. It appears that the lower concentrations of 1-MCP (0.05 ppm) more strongly inhibit physiological processes compared to the higher concentrations. The fruits treated with low concentrations of 1-MCP showed reduced intensity of weight loss, as well as slower growth of total soluble solids during the storage. On the other hand, it seems that 1-MCP has no significant effect on the firmness of the fruit flesh, since the best results in this regard have shown untreated fruits. In this regard, it is necessary to study the impact of 1-MCP treatment on cherry fruits after the storage and prior to referral to the market. Indeed, research needs to be extended to other cherry cultivars that are present in our production area.

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## AGRONOMIC EFFICIENCY OF FERTILIZATION AT DURUM WHEAT UNDER CONTRAST CLIMATE CONDITIONS

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### Abstract

The agronomic efficiency for nitrogen and phosphorus fertilization at durum wheat varieties „Progress” was studied in a long-term fertilizing experiment in Institute of field crops – Chirpan, Bulgaria. The investigation was established in two field crops rotation cotton – durum wheat under rain conditions for the period 2005 – 2011. The studied fertilizing systems were: single nitrogen (N) and single phosphorus (P<sub>2</sub>O<sub>5</sub>) fertilization in rates 0; 40; 80; 120 and 160 kg N or P<sub>2</sub>O<sub>5</sub> per hectare, and combined nitrogen-phosphorus fertilization in rates: 1). N<sub>80</sub>P<sub>80</sub>; N<sub>120</sub>P<sub>80</sub>; N<sub>160</sub>P<sub>80</sub>; 2). N<sub>80</sub>P<sub>120</sub>; N<sub>120</sub>P<sub>120</sub>; N<sub>160</sub>P<sub>120</sub>; 3). N<sub>80</sub>P<sub>160</sub>; N<sub>120</sub>P<sub>160</sub>; N<sub>160</sub>P<sub>160</sub>. Nitrogen fertilization in the form of NH<sub>4</sub>NO<sub>3</sub> was applied before sowing (1/3 of the rate) and at early spring (2/3 of the rate). The phosphorus fertilization was done before sowing in the form of triple superphosphate. According hydrothermal conditions during the wheat vegetation three of the experimental years (2005, 2007 and 2009) were classified as dry and hot. The hydrothermal conditions of the three other experimental years were close to the long term average norms of temperature and rainfall for the region.

It was established that climate conditions during the growing season were the key determinant factor for the agronomic efficiency for nitrogen in wheat. Maximum value of 27.6 kg grain kg N<sup>-1</sup> was obtained when nitrogen N<sub>80</sub> was combined with P<sub>80</sub>. Agronomic efficiency for phosphorus was significantly lower than AE<sub>N</sub>. Applying of phosphorus alone in rates higher than 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was inefficient at durum wheat, apart from the low content of available phosphates in the soil.

**Key words:** agronomic efficiency, fertilizing, durum wheat

### Introduction

Agriculture is among the sectors most directly exposed to climate variability and change, with consequences for food production and food security (Fuhrer, 2006). Because agricultural systems are often constrained by either temperature or precipitation, their sensitivity depends on the conditions under which they operate today. If a system operates near its optimum, any deviation from the ‘norm’ will limit growth and yield (Gregory and Ingram, 2008). Nitrogen and phosphorus fertilization plays a central role for improving yield in wheat and these two nutrients represent a significant cost of production for the grower (Batten, 1992; Delogua et al., 1998). They may also have environmental impacts through nitrate leaching, use of fossil fuels for manufacture and application, N<sub>2</sub>O emissions associated with denitrification and high N and P use efficiency is desired to protect ground and surface waters (Foulkes et al., 2009). Nitrogen and phosphorus are the most crucial nutrients which affect the assimilate production and distribution and affecting directly or indirectly the grain yields (Arduini et al., 2006). Nitrogen can influence the leaf area development and maintenance as well as photosynthetic efficiency and dry matter partitioning to reproductive organs (Gastal and Lemaire, 2002). In

addition, P affects the grain number and yield of wheat (Elliot et al., 1997) and diminishes biomass accumulation in a different fashion than N (Batten, 1992).

The relationship between grain yields and fertilizing rates usually are discussed as yield efficiency or agronomic efficiency. This relationship represents the yields increased per unit of applied nitrogen or other nutrients (Below, 1995; Pervaaz, 2004). There are many factors that can affect the grain yields and efficiency of nutrients of durum wheat including genotype, temperature, rainfall and fertilization (Borras et al., 2007; Miralles and Slafer, 2007). From all of them, the year conditions and fertilizing levels are usually the most important for determining of the agronomic efficiency (Delogua, 1998).

The aim of this study was to analyze the effects of different N and P fertilizer rates and their interaction on the agronomical efficiency of durum wheat grown under contrast hydrothermal conditions.

### Materials and methods

A fertilizing experiment with durum wheat variety Progress were conducted under field conditions during 2005 - 2010 growing seasons at the Institute of Field Crops - Chirpan on a eutric vertisols (FAO). The studied fertilizing systems were single nitrogen (N) and phosphorus ( $P_2O_5$ ) rates 0; 40; 80; 120 and 160 kg ha<sup>-1</sup>, and combined nitrogen-phosphorus fertilization in rates: 1). N<sub>80</sub>P<sub>80</sub>; N<sub>120</sub>P<sub>80</sub>; N<sub>160</sub>P<sub>80</sub>; 2). N<sub>80</sub>P<sub>120</sub>; N<sub>120</sub>P<sub>120</sub>; N<sub>160</sub>P<sub>120</sub>; 3). N<sub>80</sub>P<sub>160</sub>; N<sub>120</sub>P<sub>160</sub>; N<sub>160</sub>P<sub>160</sub>. The used fertilizers were ammonium nitrate and triple superphosphate. In the present study were used data of two contrast parts of growing seasons According hydrothermal conditions two years (2007 and 2009) were classified as dry and hot. The other two years (2009 and 2010) were characterized with hydrothermal conditions close to the 23-year average of temperature and rainfall. The agronomic efficiency was determined as the ratio (YF – YC)/FR, where: YF is grain yield at given fertilizing rate, YC is grain yield without N and/or P fertilization, and FR is the fertilizing rate (Guarda et al., 2004).

### Results and discussion

The obtained results show that agronomic efficiency was decreased with increasing of the nitrogen or phosphorus rates (Table 1). One kilogram applied nitrogen under favourable hydrothermal conditions was increased the durum wheat yields by 25.8 – 14.8 kg grain.

Table 1. Agronomic efficiency of nitrogen (AE<sub>N</sub>) and phosphorus (AE<sub>P</sub>) at durum wheat in dependence of the climate conditions

Year N rate	AE <sub>N</sub>		Year P rate	AE <sub>P</sub>	
	Favourable	Unfavourable		Favourable	Unfavourable
N <sub>40</sub>	25.8	9.0	P <sub>40</sub>	2.8	7.3
N <sub>80</sub>	20.4	6.8	P <sub>80</sub>	2.5	3.8
N <sub>120</sub>	19.5	7.8	P <sub>120</sub>	1.0	-
N <sub>160</sub>	14.8	2.9	P <sub>160</sub>	-	-
<i>Average</i>	<i>20.1</i>	<i>6.6</i>	<i>Average</i>	<i>2.1</i>	<i>5.6</i>

In years with unfavourable conditions average  $AE_N$  at durum wheat was 3-fold slower, than  $AE_N$  in favourable years. The obtained additional grain yield per kilogram fertilized nitrogen was varied from 9 kg at rate  $N_{40}$  to 2.9 kg at rate  $N_{160}$ , and then high nitrogen fertilization had very little effect on the grain yields.

Table 2. Agronomic efficiency of nitrogen at durum wheat under different phosphorus fertilization and climate conditions

Fertilization	Favourable			Unfavourable		
	P <sub>80</sub>	P <sub>120</sub>	P <sub>160</sub>	P <sub>80</sub>	P <sub>120</sub>	P <sub>160</sub>
N <sub>80</sub>	27.6	19.9	25.9	7.7	12.0	11.4
N <sub>120</sub>	17.8	22.2	21.8	7.0	8.3	7.3
N <sub>160</sub>	11.8	16.2	16.2	4.1	1.8	2.6
<i>Average</i>	<i>19.1</i>	<i>19.4</i>	<i>21.3</i>	<i>6.3</i>	<i>7.4</i>	<i>7.1</i>

Agronomic efficiency of P ( $AE_P$ ) was significantly lower than  $AE_N$ . Its values under unfavourable conditions were higher at rates P<sub>40</sub> and P<sub>80</sub>. Applying phosphorus alone in rates higher than 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was inefficient at durum wheat, apart from the low content of available phosphates in the soil.

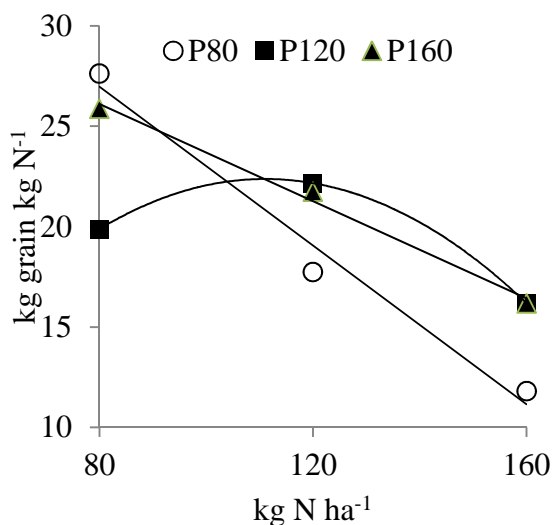


Figure 1. Agronomical efficiency of N ( $AE_N$ ) at different P fertilization under favourable hydrothermal conditions

Climate conditions during the growing season were the main determinative factor for the agronomic efficiency of applied nitrogen (Table 2). Its effect on the  $AE_N$  was similar to the effect of alone nitrogen fertilization. Combination N and P fertilization resulted in weak effect on the grain productivity of one kilogram nitrogen. Under favourable conditions among NP rate, maximum  $AE_N$  were obtained when N<sub>80</sub> was applied in combination with P<sub>80</sub>. Unfavourable climate conditions reduced obtained maximum  $AE_N$  at durum wheat from 27.6 to 12.0 kg grain per kg applied N.

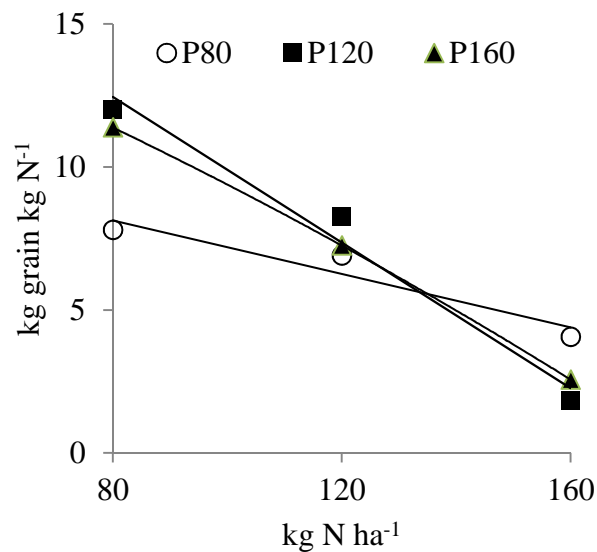


Figure 2. Agronomical efficiency of N ( $AE_N$ ) at different P fertilization under unfavourable hydrothermal conditions

Agronomic efficiency of nitrogen showed negative relationship with increase in N rates, independently of the applied phosphorus (Fig. 1 and 2). It was observed exception of this relationship when N rates were combined only with 120 kg  $P_2O_5$  ha<sup>-1</sup>.

### Conclusion

Climate conditions during the growing season were the main determinative factor for the agronomic efficiency of applied nitrogen in durum wheat. Maximum  $AE_N$  27.6 kg grain/kg N were obtained when fertilizing rate  $N_{80}$  was applied in combination with  $P_{80}$ .

Agronomic efficiency of P ( $AE_P$ ) was significantly smaller than  $AE_N$ . Applying of phosphorus alone with rates higher than 80 kg  $P_2O_5$ /ha was inefficient at durum wheat, apart from the low content of available phosphates in the soil.

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## PHOSPHORUS AND POTASSIUM AVAILABILITY CHANGE BY LIMING OF ACID SOILS

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### Abstract

Soil acidification results with the chemical changes in soil, especially with availability changes of P, K, Ca, Mg, essential and potentially toxic heavy metals. The aim of this paper was to determine the influence of acid soils liming to phosphorus and potassium availability change and to determine plant response. Liming and fertilization pot experiment of alfalfa cultivation on acid soils was set up with two types of acid soils with different texture, Silt loam (SiL) and Silty clay loam (SiCL) in a year 2009 and 2010. Ten liming and fertilization treatments were applied in four repetitions. Soil was sampled and analysed after first and second year of investigation and plant material was sampled in three cuttings at the beginning of blooming stage in each year and analysed. Results showed significant increase of soil pH values impacted by liming treatments. Soil pH increment by liming significantly increased phosphorus availability from 1,8 till 4,9 mg/kg per t/ha CaCO<sub>3</sub> and potassium availability from 1.3 till 1.5 mg/kg per t/ha CaCO<sub>3</sub> in both soils. Mineral and organic fertilization resulted with the same trend and expectedly raised phosphorus and potassium availability in soil. Furthermore, liming as well as mineral and organic fertilizer rates impacted on phosphorus and potassium concentrations increment in alfalfa leaf and stalk. Concentration increment in leaf dry matter was increased by liming from 31.1% till 38.5% for phosphorus and 20,2 % till 35.8% for potassium. Significant increment of phosphorus and potassium concentration was recorded for alfalfa stalk as well. Therefore, liming significantly increased phosphorus and potassium availability in the soil and their transfer into aboveground plant organs.

**Key words:** acid soils, liming, soil availability, plant concentrations

### Introduction

Soil acidification is a slow, continuous natural process resulting in acid soils being common in areas where soil development continued for long, geological periods of time and under climatic conditions which rainfall exceeds evapotranspiration (Rengel, 2002). Soil acidification process may be accelerated by intensive agricultural production with fertilization as one of the main factors of intensification. The process of soil acidifications is aided by water leaching base cations to lower horizons and acid soils are widespread in the eastern Croatia (Loncaric, 2006).

Soil reaction affects the soil chemical properties in wide range, especially soil nutrients mobility and plant availability. Furthermore, soil acidity and elemental toxicities or deficiencies associated with it, affects crops growth and restricts yields throughout the world (Rengel et al., 2003). Acid soils are usually excessive in soluble Al and Mn and deficient in P, Ca Mg and Mo, that may cause their reduced uptake and lead to nutrient imbalances in plants (Clark and Baligar, 2000). Consequently, in conditions of excessive soil acidity, numerous negative effects are present like hydrogen ions toxicity, toxicity of aluminum and manganese ions, phosphorus and molybdenum deficiency, reduction of microbiological

activity and increased heavy metals availability. These unfavourable chemical properties of acid soils represent one of the main factors limiting the field crops yield.

Therefore, liming is an effective ameliorative practice that may be integrated in the overall acid soil management program to attain and maintain a suitable pH range for near-optimal crop growth and yield potential (Viscarra Rossel and McBratney, 2000). Correcting soil pH by liming can influence plant nutrient availability and plant yield and quality. Thus the lime application is common practice in amelioration of acid soils in Croatia. However, increase of soil pH can also result with negative effects, such as reduction of manganese and micronutrients availability and increase of soil organic matter decomposition. Therefore, the aim of this paper was to determine the influence of acid soils liming to phosphorus and potassium availability change and to determine plant response.

### **Material and methods**

The pot experiment was conducted in field conditions, in eastern part of Croatia with two different acid soils in heavy and light textural class to investigate liming and organic fertilization effects on soil properties change as well as alfalfa mineral composition. Heavily acid silt loam soil (SiL) and extremely acid silty clay loam soil (SiCL) was set up in plastic pots of 20 L volume with 25 cm depth and 0.09 m<sup>2</sup> of soil surface. Acid soils were treated with 10 liming and organic fertilization treatments in four repetitions using alfalfa as the indicator plant. Lime material was added in two rates: 7.5 and 15 t/ha of Mischkalk lime material (65% CaO, ENV = 82.5) as well as organic fertilization: 20 and 40 t/ha cattle manure and mineral fertilization 100:200:300 kg/ha N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O. Liming and fertilization treatments were control, mineral fertilization (MF), single (OF) and double organic fertilization (2 OF), single (Ca) and double liming (2 Ca), single liming and single organic fertilization (Ca OF), single liming and double organic fertilization (Ca 2 OF), double liming and single organic fertilization (2 Ca OF) and double liming and double organic fertilization (2 Ca 2 OF).

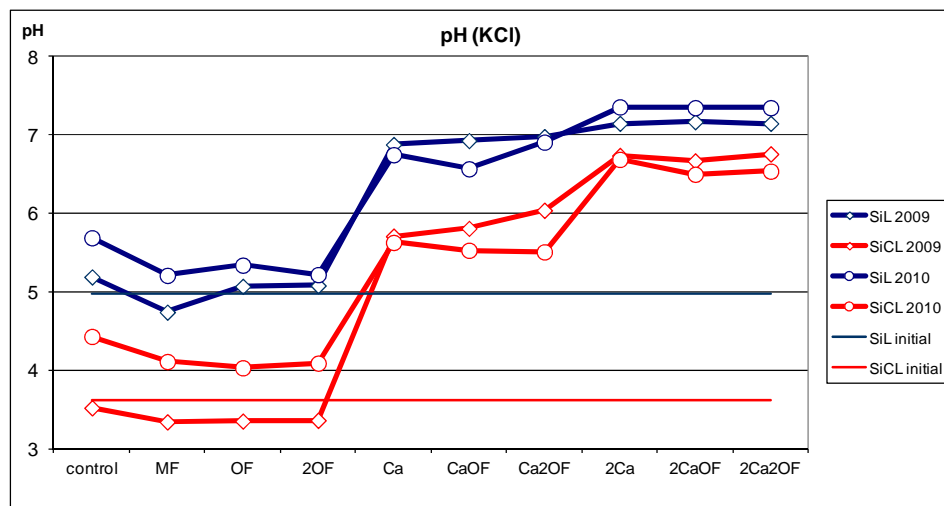
In order to collect soil data, soil samples were analysed before treatments and after first and second vegetation, in a year 2009 and 2010. During each year three cuttings of alfalfa were sampled and analysed to determine mineral composition and nutrient removal. Soil analyses included commonly used methods for soil fertility control in Croatia: pH (H<sub>2</sub>O and M KCl, 1:5 v/v) (ISO, 1994), plant available phosphorus and potassium by ammonium-lactate extraction (Egner et al., 1960). Concentration of AL-P<sub>2</sub>O<sub>5</sub> was measured by UV-spectrophotometer and concentration of AL-K<sub>2</sub>O was determined by flame atomic absorption spectroscopy. Alfalfa leaf dry matter samples were wet digested by mixture of sulphate acid and 4 % of perchloric acid and the K concentration was measured by atomic absorption spectrometer. The P leaf concentration was measured by UV-spectrophotometer. Data were statistically analyzed by ANOVA and treatment means were compared by LSD test at 0,05 probability level using Microsoft Excel and SAS 9.1 Software.

### **Results and discussion**

Results showed significant increase of the soil pH values as well as AL-P<sub>2</sub>O<sub>5</sub> and AL-K<sub>2</sub>O soil concentrations impacted by liming treatments. Soil pH reaction was significantly influenced by liming as well as mineral and organic fertilization. Results showed significant increase of soil reaction from pH(KCl) 5.69 till 7.36 in SiL soil and from pH(KCl) 4.43 till 6.54 in SiCL soil (Graph 1). In average, single liming rate raised soil pH(KCl) value for 1.53 pH units and double liming rate raised soil pH(KCl) for 2.36 pH units. In the opposite, mineral and organic fertilization decreased soil pH for average 0.4 pH units. These results were expected since rates of applied mischkalk as liming material (CCE 116.04% and ENV



82.56%) was equal to doses of 8.7 and 17.4 t/ha of pure CaCO<sub>3</sub>. Mesi et al. (2003) raised soil pH from initial pH 3.8 till pH 5.31 and till pH 5.52 by application of 10 t/ha and 20 t/ha CaCO<sub>3</sub> at the end of the first year of experiment. Rastija et al. (2008) recorded soil pH increase for 1.2 pH units as a result of application of 10 t/ha of Carbocalk (ENV 69%) in the first year of experiment.



Graph 1. Dynamics of soil pH change in 2009 and 2010.

In SiL soil initial AL-P<sub>2</sub>O<sub>5</sub> concentration was 172.4 mg/kg and considerably lower, 102.2 mg/kg in SiCL soil. Mineral fertilization expeditedly increased AL-P<sub>2</sub>O<sub>5</sub> concentration compared to control in both years, but without statistical significance. Double level of organic fertilization significantly increased phosphorus availability. The single liming level application significantly increased phosphorus availability in both years compared to control and in second year of investigation compared to mineral fertilization treatment. Double liming level impacted on additional and statistically significant AL-P<sub>2</sub>O<sub>5</sub> increment compared to single liming level on both soils, but only in a year 2010 (Table 1).

Table 1. Impact of liming and fertilization on AL-P<sub>2</sub>O<sub>5</sub> (mg/kg) in 2009 and 2010.

treatments	AL-P <sub>2</sub> O <sub>5</sub> mg/kg – 2009.			AL-P <sub>2</sub> O <sub>5</sub> mg/kg – 2010.		
	SiL	SiCL	average	SiL	SiCL	average
control	179.0 c	100.0 f	139.50 G	135.17 g	92.67 f	113.92 H
MF	194.7 bc	122.0 ef	158.38 GF	148.50 fg	103.00 ef	125.75 G
OF	200.2 bc	129.0 def	164.63 EF	152.80 ef	107.33 def	129.92 FG
2 OF	244.0 a	149.75 cde	196.88 CD	165.00 e	114.67 de	139.83 F
Ca	231.5 ab	138.33 cde	184.92 DE	190.82 d	118.33 d	154.58 E
Ca OF	248.5 a	170.25 bc	209.38 BCD	216.50 bc	158.33 b	187.42 C
Ca 2 OF	254.3 a	166.25 bcd	210.29 BC	222.50 bc	160.33 b	191.42 BC
2 Ca	234.5 a	157.33 cde	195.92 CD	214.50 c	136.00 c	175.25 D
2 Ca OF	252.7 a	201.50 ab	227.13 AB	231.32 ab	167.33 ab	199.33 B
2 Ca 2 OF	266.5 a	209.50 a	238.00 A	245.50 a	179.00 a	212.25 A
average	230.61 A	154.39 B		192.23 A	133.70 B	

The single and double liming doses increased average AL-P<sub>2</sub>O<sub>5</sub> soil concentrations from 1.8 mg/kg P<sub>2</sub>O<sub>5</sub> till 4,9 mg/kg P<sub>2</sub>O<sub>5</sub> per every t/ha of applied CaCO<sub>3</sub>. According to the results, it is possible to determine the regularity of plant available phosphorus increase with soil pH increase or with excessive soil acidity neutralization by liming. Mineral and organic fertilization resulted with the same trend and expectedly raised phosphorus availability in the soil. Haynes (1982) reported that moderately increment of soil pH by liming impacts on phosphorus availability increment in soil, while too high liming doses could result with phosphorus availability decrement in soil.

Table 2. Impact of liming and fertilization on AL-K<sub>2</sub>O (mg/kg) in 2009 and 2010.

treatments	AL-K <sub>2</sub> O mg/kg – 2009.			AL-K <sub>2</sub> O mg/kg – 2010.		
	SiL	SiCL	average	SiL	SiCL	average
control	80.95 d	130.37 c	105.66 D	60.40 d	114.07 f	87.23 F
MF	96.08 bcd	153.45 b	124.76 C	79.60 c	141.30 d	110.45 D
OF	90.68 cd	157.57 b	124.12 C	78.35 c	121.80 f	110.07 E
2 OF	102.83 abc	163.92 ab	133.37 BC	80.37 c	130.50 e	105.44 DE
Ca	109.43 abc	160.37 ab	134.90 BC	89.72 abc	150.70 c	120.21 C
Ca OF	110.63 abc	167.82 ab	139.22 AB	91.12 abc	152.95 bc	112.04 BC
Ca 2 OF	103.63 abc	161.95 ab	132.79 BC	87.70 bc	131.53 e	109.62 D
2 Ca	112.48 ab	168.22 ab	140.35 AB	97.20 ab	158.05 abc	127.62 ABC
2 Ca OF	114.40 ab	170.57 ab	142.49 AB	100.95 ab	160.37 ab	130.66 AB
2 Ca 2 OF	120.23 a	175.52 a	147.87 A	103.22 a	164.70 a	133.96 A
average	104.13 B	160.98 A		86.86 B	142.60 A	

Initial potassium availability (AL-K<sub>2</sub>O) in SiL soil was 79.0 mg/kg and in SiCL soil was 124.0 mg/kg. Liming and mineral as well as organic fertilization significantly impacted on potassium availability change in soil. In SiL soil, all liming treatments significantly increased potassium availability compared to control in a year 2009. In the same soil, in a year 2010 potassium availability was lower for all treatments, but the differences between liming and control treatment became even more expressed. In the SiCL soil, double liming level with double organic fertilization resulted with significantly the highest potassium concentration compared to control in a first year of investigation. In a year 2010 this difference became more expressed, although all the concentrations of available potassium were lower (Table 2). Treatments of single liming doses minimally increased soil AL-K<sub>2</sub>O concentrations for 1.38 mg/kg K<sub>2</sub>O, while application of double liming doses increased potassium availability for 1.46 mg/kg K<sub>2</sub>O per every t/ha of applied CaCO<sub>3</sub>. Mineral and organic fertilization showed identical trend and expectedly raised potassium availability in soil.

Furthermore, liming as well as mineral and organic fertilizer rates impacted on phosphorus and potassium concentrations increment in alfalfa leaf. Significant increment of phosphorus and potassium concentration was recorded for alfalfa stalk as well. Therefore, liming significantly increased phosphorus and potassium availability in the soil and their transfer into aboveground plant organs.

Table 3. Impact of liming and fertilization on P (%) leaf concentration in 2009 and 2010.

treatments	P % leaf – 2009.			P % leaf – 2010.		
	SiL	SiCL	average	SiL	SiCL	average
control	0.270 d	0.257 e	0.266 E	0.232 c	0.207 d	0.219 E
MF	0.300 c	0.290 cd	0.296 D	0.267 bc	0.257 c	0.262 D
OF	0.300 c	0.287d	0.296 D	0.270 bc	0.260 c	0.265 D
2 OF	0.310 bc	0.301 bcd	0.307 BCD	0.282 abc	0.273 bc	0.278 CD
Ca	0.310 bc	0.300 bcd	0.307 BCD	0.300 ab	0.273 bc	0.287 BCD
Ca OF	0.310 bc	0.303 bc	0.309 BC	0.307 ab	0.293 abc	0.300 ABC
Ca 2 OF	0.300 c	0.293 bcd	0.299 CD	0.310 ab	0.303 ab	0.307 ABC
2 Ca	0.330 ab	0.307 b	0.317 B	0.312 ab	0.293 abc	0.303 ABC
2 Ca OF	0.330 ab	0.305 bc	0.316 B	0.317 ab	0.317 a	0.317 AB
2 Ca 2 OF	0.340 a	0.325 a	0.331 A	0.330 a	0.327 a	0.328 A
average	0.310 A	0.300 B		0.293 A	0.280 A	

As it was expected, mineral and organic fertilization significantly impacted on phosphorus leaf concentration increment compared to control treatment on the both soils in 2009. and 2010. Significance of a single liming level application on phosphorus leaf concentration was determined in comparison with control treatment in the both investigation years and on both soils. Increment of phosphorus leaf concentration under impact of a double liming level was not statistically significant compared to single liming level impact on both soils and in both years (Table 3).

Bergman (1992) specified optimal nutrient status in aboveground plant mass of alfalfa at the beginning of flowering stage. Values are expressed on the basis of dry matter mass, and range from 0.30 – 0.60% P and 2.50 – 3.80% K. Determined concentrations of alfalfa leaf dry matter mineral composition on conducted experiment were matching the mentioned values, where liming doses resulted with expressed effect of phosphorus concentration increment in alfalfa leaf dry matter as a result of soil pH increase by liming. By alfalfa growing on two acid soils, phosphorus leaf concentration was increased from 15.4% till 31.1% with single liming treatments and from 19.2% till 38.3% with double liming treatments.

Table 4. Impact of liming and fertilization on K (%) leaf concentration in 2009 and 2010.

treatments	K % leaf – 2009.			K % leaf – 2010.		
	SiL	SiCL	average	SiL	SiCL	average
control	1.85 d	1.63 g	1.74 F	1.13 f	1.05 e	1.09 G
MF	2.03 cd	1.96 f	2.00 E	1.28 e	1.18 d	1.23 F
OF	2.05 d	2.05 ef	2.05 DE	1.30 de	1.17 d	1.23 F
2 OF	2.12 bc	2.11 de	2.12 CD	1.34 cde	1.24 cd	1.29 E
Ca	2.16 bc	2.14 cd	2.15 C	1.35 cde	1.26 bcd	1.31 DE
Ca OF	2.20 abc	2.20 bc	2.20 BC	1.39 bcde	1.28 bcd	1.33 CD
Ca 2 OF	2.21 abc	2.17 cd	2.19 BC	1.41 abcd	1.34 abc	1.37 C
2 Ca	2.27 ab	2.24 abc	2.25 AB	1.43 abc	1.33 bc	1.38 BC
2 Ca OF	2.28 ab	2.27 ab	2.28 AB	1.47 ab	1.37 ab	1.42 B
2 Ca 2 OF	2.35 a	2.31 a	2.33 A	1.51 a	1.45 a	1.48 A
average	2.15 A	2.11 A		1.36 A	1.27 B	

Expectedly, mineral and organic fertilization significantly increased potassium concentration in alfalfa leaf dry matter compared to control treatment on SiCL soil in 2009 and on both soils in 2010. Application of single liming doses resulted with significant impact on

potassium leaf concentration increment compared to control treatment on both soils in 2009 and 2010. Double liming level impacted on more intensive potassium leaf concentration increment in comparison with control treatment, but on both investigated soils in 2009 and 2010 potassium leaf concentration increment was not statistically significant compared to increment accomplished by single liming doses (Table 4).

In the second year of experiment, significantly lower potassium leaf concentrations were recorded in comparison with the first year of experiment. Application of single liming doses increased average potassium leaf concentrations from 20.2% till 23.6% and double liming doses increased average concentrations from 22.6% till 26.6%.

### Conclusions

Liming significantly raised soil pH in both soils and the final effect of liming on soil pH was depending on initial soil pH. In the opposite, mineral and organic fertilization impacted on soil acidity increase and partially neutralised liming effect on soil pH. Application of liming material increased phosphorus availability in soil and availability increment was higher in soils with higher initial phosphorus availability. Organic and mineral fertilization expectedly impacted on soil phosphorus availability increase. Liming also impacted on potassium availability increase in soil. Organic fertilization did not have significant impact on potassium availability change. Increment of liming material doses and organic fertilization resulted with phosphorus and potassium concentrations increase in alfalfa leaf dry matter and stalk as well.

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**THE EFFECT OF REGALIS CONCENTRATION ON THE SHOOT CHARACTERISTICS OF PEAR VARIETY PASSE CRASSANE**

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**Abstract**

The effect of Regalis (Prohexadione Calcium) on shoot growth was evaluated on pear variety Passe Crassane. Three different doses of Prohexadione Calcium were applied: 50 ppm, 100 ppm and 150 ppm. Treatments were compared with control without treatments. 4 foliar treatments were applied with the first one 5 days after petal fall and the others every 10 days after. Significant differences between the control and the treatments were observed.. Final shoot length was generally reduced by treatments by 14.5% to 22% as compared to untreated trees. The length of the shoots was not significantly different between 50 ppm and 100 ppm.

**Keywords:** Regalis, pear, shoot length.

**Introduction**

Several techniques have been employed to avoid excessive shoot growth as different types of dwarfing rootstocks, dormant and summer pruning, root pruning, root restriction, stem girdling or sawing, limb bending, breaking or reducing fertilization and irrigation. Alternatively or additionally, plant regulators may be employed for the reduction of shoot growth (Asin, Mass, Musacchi, Pages, Sansavini, Sugar, Vilardell.).

Plant growth regulators that inhibit the development and growth of shoots have been used to reduce the amount of shoot growth and subsequently increase yield. They can be well integrated into orchard production systems. Gibberellin biosynthesis inhibitors have a key role in cell elongation (5,8). The most used growth retardant is the Prohexadione-calcium (Regalis) that has low toxicity and persistence in the plant. The inhibitory effect of Prohexadione-calcium (Regalis) is based on the formation of growth-active gibberellin (8) that leads to a reduction of longitudinal shoot growth.

Trials with Prohexadione-calcium to control vegetative growth of apple, pear and plum trees were demonstrated by other authors (1,8).. The objective of this research was to quantify the efficacy Prohexadione-calcium (Regalis) at three different concentrations, on three treatment times, on pear cultivar Passe Crassane.

**Materials and Methods**

Investigations of Prohexadione-calcium (Regalis) effect on pear tree cv. Passe Crassane were carried out at the region of Peja, municipality of Kline, Republic of Kosova. The orchard was planted in the spring of 2009. Planting distance were 3 x 1.5 m. Trees were trained as slender spindle. A randomized complete block with four replications, four treatments and two trees per experimental unit was used as the experimental design.

Regalis ranging of treatments were as follow:

Control without treatment

Treatment with 50 ppm (a.i.) Regalis

Treatment with 100 ppm (a.i.) Regalis

Treatment with 150 ppm (a.i.) Regalis

The first treatment was applied 7 days after the petal fall, the second 10 days after the first treatment, and the third 10 days after the second treatment

The length of shoots, the number of the nodes, the length of internodes and number of leaves of new growth (current growing season) on 15 randomly selected extension shoots (selected randomly on each tree) was measured at the end of growing season, on 10 October. Differences between means of each treatment were analyzed by the Duncan and Dunnet's multiple range test ( $P < 0.05$ ).

### Results and Discussion

Vegetative growth is the parameter the most obviously affected by Prohexadione-calcium (Regalis) applications. The inhibitory effect of the Prohexadione-calcium (Regalis) formation of growth-active gibberellin (8) leads to a reduction of longitudinal shoot growth.

**Table 1** summarizes the results of several experiments with 'Passe Crassane', in which the effects of different dosages of application had been evaluated. Final shoot length was generally reduced by treatments by 14.5% to 22 % as compared to untreated trees. The length of the shoots was not significantly different between 50 ppm and 100 ppm. Our results that the successful effects of Regalis use in pear was difficult to achieve confirmed the results of other researchers (3). The treatment of the pome fruit trees with Regalis both a single application of  $250 \text{ g ha}^{-1}$  (a.i.) or a split treatment with  $2 \times 125 \text{ g ha}^{-1}$  (a.i.) led to an average reduction of shoot growth by approximately 40% (9).

Tab1. Effect of Prohexadione –calcium (Regalis) on the length of shoot (cm)

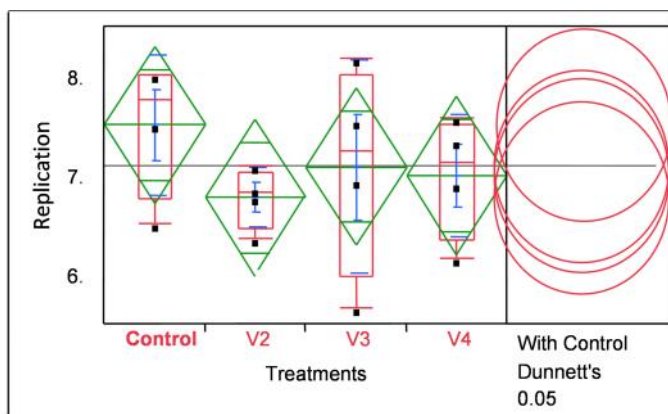
Treatments	Means
V0(control )	28.173 a
V1	23.953 b
V2	23.992b
V3	21.396b

\* Separation by Duncan's multiple range tests, at  $P < .05$

Tab 2 shows that number of nodes was not significantly differ between non-treated trees and treated trees, while the increase in shoot length in absolute terms was different between treatments. This means that the treatment with Regalis inhibits growth of the shoots but did not prevent the formation of the buds. For this reason the average number of the buds is equal in treated trees and untreated ones.

Tab2. The mean number of nodes per shoots

Treatments	Means
V0(control)	8.0107
V1	7,278
V2	7,588
V3	7,501

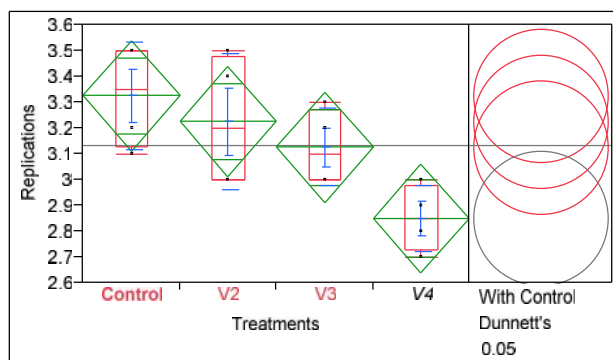


Evaluation of the data from Table 1 with Table 3 shows that the increase of the shoot length is proportional to the increase of internodes length. This means that the buds formed on the trees treated with Regalis have less competition from the apical dominance of the growing shoot. Consequently, the possibility of their differentiation in flowering must be greater. This has been confirmed by other authors. Excessive vigor could be considered to competitively inhibit flowering through hormonal control (6).

Tab3. The mean length of internodes (cm)

Treatments	Means
V0	3.325 a
V1	3.225a+
V2	3.125a
V3	2.85b

\* Separation by Duncan's multiple range tests, at  $P < .05$



As can be seen in Tab.4 the numbers of the leaves were almost equal in all the treated and untreated trees.

Analyzing Tables 1 2 3 and 4 we found that f Regalis has not significantly affect the number of nodes and consequently the number of buds and the number of leaves. This confirms that only shoot growth and internodes length may be controlled by Regalis application.

Tab.4 the mean number of leaves per shoot

Treatments	Means
V0	17.3121a
V1	17.3458a
V2	16.7142a
V3	16.5113a

So, the use of Regalis reduces the apical dominance of the growth of the shoots and maybe promotes the differentiation of flowers on the lateral buds.



(A)



(B)

Figure1. A-treatment with 150 ppm Regalis; B-Without treatment

### Conclusions

Final shoot length was generally reduced by treatments by 14.5% to 22 % as compared to untreated trees. The length of the shoots was not significantly different between 50 ppm and 100 ppm.. To define the right application doze and the timing more researches and experiments are required.

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## GGE BIPLLOT ANALYSIS OF WHEAT MEAN PERFORMANCE AND STABILITY AT DIVERSE LOCATIONS IN REPUBLIC OF MACEDONIA

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### Abstract

Wheat is the most important cereal crop in Republic of Macedonia. Development of genotypes that will have high grain yield with stable performance in different growing conditions is of paramount importance. The objective of this research was to evaluate and to quantify the magnitude of the genotype x environment interaction effects on wheat grain yield and to determine the winning genotype for the test locations. Ten wheat genotypes were tested at three locations (Skopje, Strumica and Prilep) for two years (2006 and 2007). The grain yield data for each location were subjected to the GGE biplot analysis. This analysis depicted the adaptation pattern of genotypes at different locations and discrimination ability of testing locations. Out of the three locations, Prilep was identified as the most discriminative and representative location. The genotype Bt 04-073 had the highest overall mean yield, and an average stability over different locations. In each location, Radika and Bt 04-073 were the closest to the “ideal” genotype, followed by Bt 04-024. Those genotypes can be recommended for production in wheat growing regions in Republic of Macedonia.

**Key words:** wheat, genotype x environment interaction, GGE biplot, grain yield, stability analysis

### Introduction

In the Republic of Macedonia, wheat is the most important cereal crop, grown on approximately 84181 ha in the last five years. Grain yield of cultivated genotypes in Macedonia is insignificantly higher (3056 kg ha<sup>-1</sup>) than the global average yields (3010 kg ha<sup>-1</sup>), but compared with the highest yield (7772 kg ha<sup>-1</sup>), it is far below this value (FAOSTAT, 2011). In order to mitigate this, different improved cultivars are continually created or introduced. Those cultivars are being evaluated at different locations to test their performance and to identify the best genotypes in specific environments. The genotype × environment (GE) interaction usually complicates the selection for improved yield (Sabaghnia et al., 2013). According to Rodriguez et al. (2002) in the cases when the magnitude of GE is large it impedes the selection of stable genotypes and the selection advancement is slow.

Ceccarelli (1989) claimed that adaptation in crop plants corresponds to yield stability over time and environments. Considering this, when unpredictable GE interaction is present, cultivar evaluation must be carried out in multiple locations in order to fully test the target environment (Cooper et al., 2007). Consequently, for assessing genotypic value and cultivar’s stability for yield performance, multi-environmental trial (MET) data are required. The main purpose of MET is to identify superior cultivars which could be recommended to farmers and to determine sites that best represent the target environment (Yan and Hunt, 2001).

The genotype performance across different environments could be difficult to determine without the help of graphical display of the data (Yan et al., 2001). Yan and Hunt (2001) proposed a GGE biplot that allows visual examination of the GE interaction pattern of the data. GGE biplot refers to the genotype main effect (G) and the genotype x environment interaction (GE), the main two sources of variation that are important to cultivar evaluation. It can be used to identify superior cultivars and test environments that facilitate identification of such cultivars. The detection of genotypes that have the highest yield across a number of environments could be useful to breeders and producers (Kaya et al., 2006). Thus, information on wheat cultivar stability in different environments, along with high yield may be helpful in selection of genotype(s) that have the best performance in favorable environments, but also maintain satisfactory yields under poor management.

The GGE-biplot methodology was used in this study (i) to graphically summarize the effects of genotypes (G) and genotype x environment (GE) interaction on yield performance of 10 bread wheat genotypes tested across 3 locations, (ii) to facilitate visual comparison among genotypes and environments and (iii) to determine if wheat growing regions in Macedonia might be divided into distinct MEs.

### **Materials and methods**

The study was carried out in 2006 and 2007 in three different locations in Republic of Macedonia (Skopje, Prilep and Strumica). A set of 10 bread wheat genotypes (Radika, Bt 04-005, Bt 04-024, Bt 04-030, Bt 04-040, Bt 04-073, Bt 04-081, Bt 04-082, IJZK 16/99, MO 11/4) was used as experimental material (Table 1). Experimental layout was a randomized complete block design with five replications in each location. Each plot consisted of eight rows of 5 meter length. Between row distance was 12.5 cm. In order to eliminate side effects, data on grain yield were taken from the middle six rows of each plot. At harvest grain yield was determined for each genotype at each test location. The GGE biplot analysis was performed using R statistical software.

### **Results and discussion**

GGE denotes genotype main effect (G) plus genotype by environment interaction (GE). For appropriate genotype evaluation (Yan, 2002), these two sources of variation that are relevant to genotype evaluation must be considered simultaneously, not alone or separately.

The vector view of the GGE biplot (Figure 1) presents a summary of the interrelationships among the locations. The test locations are connected to the biplot origin by lines, called environment vectors. The cosine of the angle between the vectors of two locations approximates the correlation between them (Kroonenberg, 1995; Yan, 2002). The distance between two locations represents their dissimilarity in discriminating the genotypes. Thus, the three locations fell into three different groups. The large angle between them implies poor correlation between these locations (Figure 1).

Table1. Genotype code and name of 10 bread wheat genotypes with their average grain yield across three locations

Genotype code	Genotype name	Grain yield (kg ha <sup>-1</sup> ) per location			Average yield (kg ha <sup>-1</sup> )
		Skopje	Strumica	Prilep	
1	Radika	7.60	6.65	4.70	6.32
2	Bt 04-005	7.70	6.05	4.10	5.95
3	Bt 04-024	7.45	6.75	4.70	6.30
4	Bt 04-030	8.00	6.40	3.80	6.07
5	Bt 04-040	8.15	5.80	4.40	6.12
6	Bt 04-073	7.90	6.95	4.60	6.48
7	Bt 04-081	7.60	5.95	3.60	5.72
8	Bt 04-082	7.60	6.30	4.30	6.07
9	IJZK 16/99	7.50	6.85	4.60	6.32
10	MO 11/4	6.90	6.90	3.60	5.80

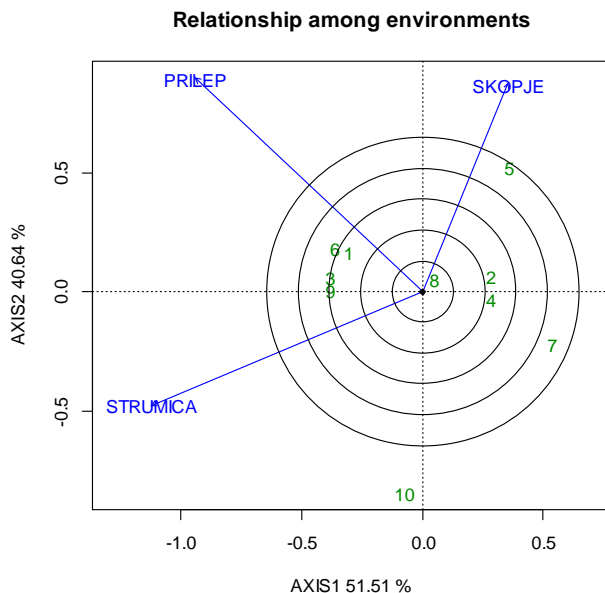


Figure 1. GGE biplot based on relationships among test environments.

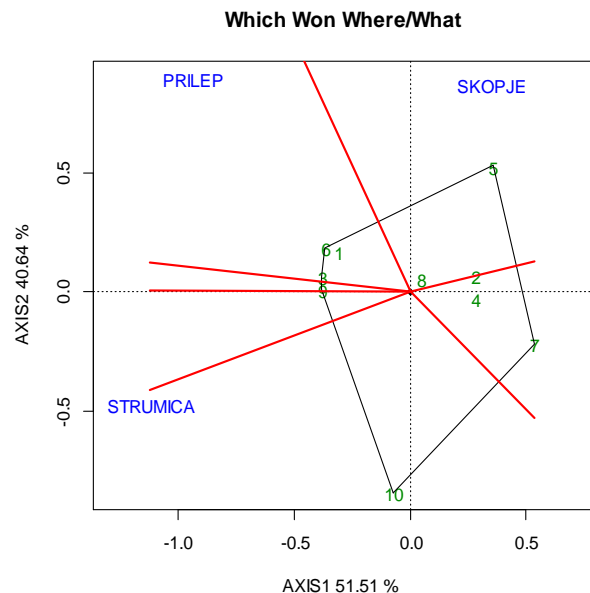


Figure 2. Which-won-where pattern of genotypes and environments.

One of the best possibilities that the GGE biplot methodology offers is showing the which-won-where pattern of a genotype by environment dataset (Figure 2). It graphically addresses important concepts such as crossover GE, mega environment differentiation, particular adaptation, etc (Yan and Tinker, 2005). The polygon is created by linking the markers of the genotypes that are the furthest away from the biplot origin such that all other genotypes are positioned in the polygon. Genotypes located on the vertices of the polygon performed either the best or the poorest in one or more environments. The vertex genotype(s) for each sector has higher (sometimes the highest) yield than the others in all environments that fall in that sector (Yan, 2002). The perpendicular lines are equality lines between adjacent genotypes on the polygon, which facilitate their visual comparison. Thus, all locations included in this

study fell into different sectors. The vertex genotype for the sector where Skopje was positioned was 5, for Prilep region was 6 and for Strumica region was genotype 10.

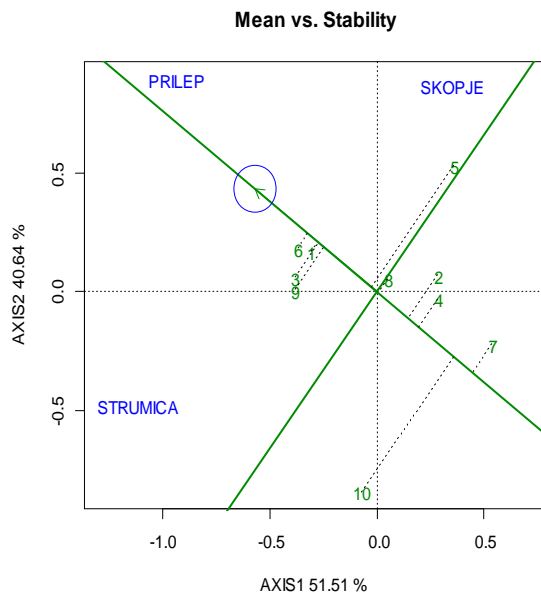


Figure 3. GGE biplot representing mean performance and genotypes stability

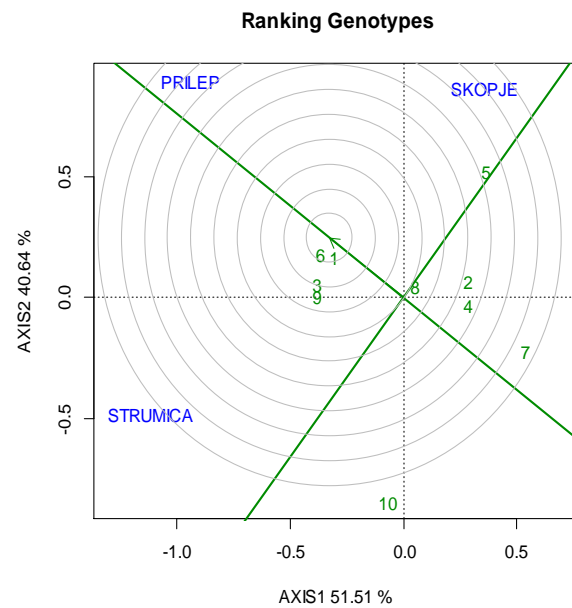


Figure 4. Genotype-focused scaling for comparison of the genotypes

The ranking of 10 genotypes based on their mean grain yield and yield stability for 3 locations is shown in Figure 3. It has been reported that when PC1 in a GGE biplot approximates the G (mean performance), PC2 must approximate the  $G \times E$  associated with each genotype, which is a measure of instability (Yan et al., 2000; Yan, 2002). The line that passes through the biplot origin and the environmental average is known as the average environment coordinate (AEC) axis and is indicated by circle, which is defined by average PC1 and PC2 scores for all environments. Projection of genotype markers onto this axis represents the mean yield of the genotypes and the arrow points to higher mean yield across environments. Thus, genotypes 6, 1, 3 and 9 had higher grain yield than the other genotypes from this dataset. The line which passes through the origin but is perpendicular to the AEC represents the status of the genotypes stability. A position in either direction away from the biplot origin, on this axis, indicates greater  $G \times E$  interaction and reduced stability (Yan, 2002). Therefore, genotypes 6, 1 and 8 were the most stable, while genotypes 5 and 10 showed more variable and the least stable performance (Figure 3). Similarly, when ranking genotypes (Figure 4), genotypes 6 and 1 and the closest to the "ideal" genotype.

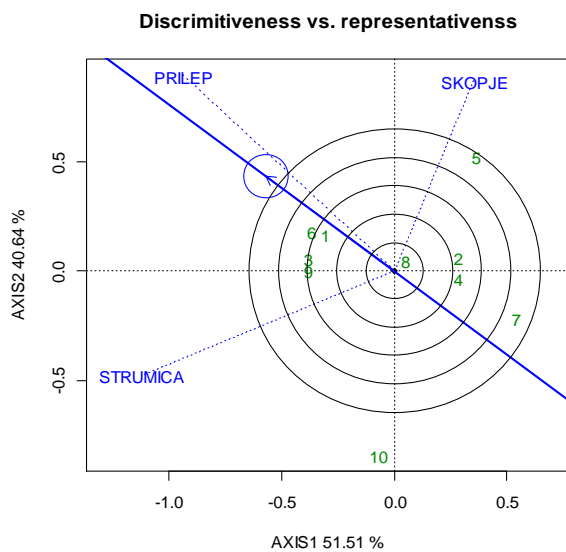


Figure 5. Discriminability and representativeness of the three locations

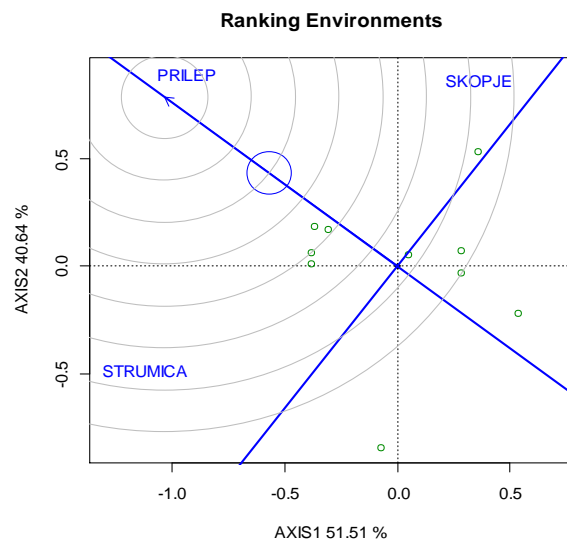


Figure 6. GGE biplot based on environment-focused scaling

The length of the environment vectors on the biplot approximates the standard deviation within each environment, which is a measure of its discriminating ability (Yan and Kang, 2003). Considering this, Prilep was the most discriminative location (Figure 5). When ranking the environments (Figure 6), Prilep was the closest to the “ideal” environment.

### Conclusions

The analyzed ten bread wheat genotypes showed high variability for grain yield. The three locations included in this study could be considered as different mega-environments for wheat testing. Genotypes 6 (Bt 04-073) and 1 (Radika) had the highest average yield and were the most stable genotypes. Genotypes 5 (Bt 04-040) and 4 (Bt 04-030) had the best performance in Skopje region, 6 (Bt 04-073) and 10 (MO 11/4) in Strumica and 1 (Radika) and 3 (Bt 04-024) in Prilep. These genotypes should be recommended for growing in these specific locations.

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**MORPHOLOGICAL DIVERSITY OF SOME OPIUM POPPY GENOTYPES  
(PAPAVER SOMNIFERUM L.)**

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**Abstract**

Cluster analysis using qualitative variables is a useful tool in estimating genetic diversity between genotypes in a germplasm collection. The objective of this study was to classify opium poppy genotypes based on several morpho-qualitative traits of the flowers, capsules and seed. The collection of 50 poppy genotypes with different origin was evaluated in 2010, on experimental field near Skopje. The classification of the genotypes was done based on Gower distance and the dendrogram was constructed using UPGMA method. Two main clusters were identified, each comprising different number of subgroups. All genotypes with white petal color were grouped in the second cluster. Genotypes with colored petals belonged to the first cluster. The origin of the genotypes had no influence on the classification. The results of this study enabled clear overview of the morphological diversity identified in the studied germplasm.

**Key words:** opium poppy, morphological diversity, qualitative traits, cluster analysis

**Introduction**

Republic of Macedonia has a very long tradition in growing opium poppy. In the nineteenth century it was one of the most important crops in Macedonia (Nikolic, 1954) and was used for opium production. Macedonian opium had the highest morphine content in the world (Bensussan, 1946), which, calculated in dehydrated opium varies between 15.9% and 17.2% (Vajic and Mikic, 1951). Today, the opium poppy is grown for its seed and capsules and morphine is extracted from the dry capsules. The production is mainly based on local landraces and one commercial cultivar and as a result, in the recent years, the need to improve existing germplasm became apparent. The renewal and upgrade of the poppy breeding program is of utmost importance.

The main goal in European poppy breeding programs in the long run was to create universal type, that will have high seed yield, suitable color (usually blue) as well as high morphine content in ripe capsule wall, which was in line with the poppy ideotype (Matyasova et al., 2011). One of the most important steps for genetic improvement of crops through conventional breeding is to study the genetic diversity available in the introduced plant/crop material (Mishra et al., 2013). To conduct any breeding program judiciously, diversity analysis based on morphological and biochemical traits is prerequisite. In opium poppy, several collections at different research institutes have been evaluated for genetic diversity. Singh et al. (2004) studied genetic divergence between 101 germplasm lines from different ecogeographical origin based on quantitative traits using multivariate and canonical analysis. They identified clusters with genotypes which had greater potential as breeding material



because they comprised high mean values for one or more component characters and high statistical distances among them. Yadav et al. (2007, a) investigated the genetic divergence in a genetically distinct opium poppy genotypes by cluster and principle component analysis. They recommended some accessions which may be used in hybridization programme to obtain desirable progeny. Yadav et al. (2007, b) also assessed the genetic divergence in a population of 20 parents and 90 F<sub>1</sub> hybrids. All genotypes were grouped into 14 clusters which indicate significant diversity among parents. As a result, considerable variation existed in their crosses. Furthermore, Brezinova et al. (2009) evaluated poppy genotypes from the world collection and observed important diversity for morphological characteristics. Several researchers evaluated the genetic diversity based on alkaloid spectrum (Shukla et al., 2010; Nemeth-Zambori et al., 2011; Stranska et al., 2013).

For establishing an effective breeding program, the genotypes in the starting collection should be grouped according to their characteristics. Therefore, the present study was undertaken with the following objectives: (a) to analyze the genetic diversity among poppy landraces and breeding lines from Macedonia and introduced poppy genotypes based on various morphological traits and (b) to determine the extent of genetic diversity for proper utilization in the breeding program.

### **Material and methods**

A total of 50 opium poppy genotypes were evaluated in randomized block design with 2 replications during 2010-2011. The experimental field was located near Skopje, Republic of Macedonia, between 42°05'N latitude and 21°23' E longitude, 402 m above sea level. The germplasm collection comprised Macedonian land races and advanced breeding lines as well as genotypes of different geographic origin (Table 1). Each plot consisted of two rows, 2 m long, with of 30 cm between rows and 5 cm within row spacing. During the vegetative growth standard agro-technical practices were applied.

The morphological traits were described according to UPOV descriptor (1999). Ten randomly selected plants per replication were marked before flowering and the observations were recorded on 14 morphological characters of the stem (hairiness between the capsule and upper stem leaf), petal (petal color, petal color intensity, presence of blotch, color of blotch, intensity of violet color of blotch), capsule (shape of base, shape of longitudinal section, depth of ribbing and dehiscence, stigmatic disk shape, surface of stigmatic disc lobes, apex of stigmatic disc lobes) and seed (color). The genotypes were clustered based on Gower's distance and the dendrogram was constructed using UPGMA method. The package "cluster" from R statistical software was used for the analyses.

Table 1. Origin and code of poppy genotypes

Origin	Genotype code
Macedonia	29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50
Turkey	12, 13, 20, 21, 22, 23, 24, 25, 26, 27, 28
Bulgaria	4, 5, 6, 7
Australia	1
Austria	2
Belgium	3
Morocco	8, 9, 10
Portugal	11
Ukraine	14
Hungary	15, 16, 17
Sweden	18
unknown	19

### Results and discussion

The poppy genotypes expressed various morphological characteristics and attempts have been made to classify them on the basis of qualitative traits. The stems of the poppy plant may be glabrous or hairy. Out of 50 genotypes, 36 had hairs on the stem, between the capsule and the upper stem leaf. The petal color in poppy can vary from white to dark violet, with different intensity. White petal color without blotch was observed in 15 genotypes. 2 genotypes had red petal color with dark violet blotch. The remaining 33 genotypes had different intensity of violet color of the petal and blotch. Brezinova et al. (2009) identified genotypes with different intensity of red petal color.

The form of the capsule is one of the most stable characteristics of the poppy. According to the capsules, the poppy genotypes can be classified in two categories: with closed or with open capsules. Both open and closed capsules can be found on the same plant. The closed capsules do not open automatically when the plant ripens and they have to be thrashed in order to collect the seeds. This trait is desirable in poppy because there is no seed loss due to capsule dehiscence. In this category belonged 42 of the evaluated genotypes, while only 8 had open capsules.

The shape of the capsule is a typical cultivar trait (Brezinova et al., 2009), but it is very unbalanced in genotypes with higher number of capsules that gradually mature. According to Borecki and Stiffel (1995), the breeding aim is a opium poppy cultivar with capsules of globular shape which contains the biggest share of big seeds. Conical shape was detected in 27 genotypes, 10 genotypes had circular capsules and the others had flattened (5), rectangular (5) and elliptic (3) capsules. The colour of the poppy seeds has many variations, but in any one capsule is almost always uniform, only the shade sometimes varies. If there is no cross-pollination, the seed colour is hereditary and remains unchanged. It wasn't possible to establish final correlation between the petal color and seed color, but in most cases the poppy with white or rose-colored flowers have white or light-colored seeds and poppies with violet or dark-colored flowers give blue or dark-colored seeds. Most of the genotypes in this study had different shade of gray color (29), 9 genotypes had white seeds, 5 had ochre color, 8 were with brown seed color and only 1 genotype had pink seed.

Based on the UPGMA method, the germplasm lines were grouped into two main clusters (Figure 1). The cluster I consisted of poppy genotypes with white flowers, while in cluster II

belonged genotypes with violet and red flowers. Each cluster comprised 2 subclusters and different number of genotypes. Cluster I consisted of 15 genotypes that were separated in two main subgroups. In the first subgroup only two genotypes were positioned (16 and 18). The second subgroup comprised 13 non-dehiscent genotypes. Except genotype 25 all other had oval lobus apexes and had white, ochra and brown seeds. For the other characters, all forms were present. The first subcluster from the cluster II contained 6 genotypes. All of them had light or medium intensity of violet color, except genotype 11 with red petal color. All genotypes in this subcluster had flat capsule base and the circular form prevailed. Only genotype 13 had rectangular capsule. In the second subcluster from the cluster II belonged genotypes with light, medium or dark violet color. Genotype 50 had red petal color. Most of the genotypes in this group had flat capsule base, capsules with shallow or medium ribbing, and all forms of capsules except flattened were present. Only three genotypes were dehiscent. The majority had conical capsules and gray seeds. Classification of germplasm lines into different groups or subgroups was independently of their origin. It was difficult to establish any relationship between the origin and clustering pattern.

The preliminary characterization of opium poppy genotypes gives a clear overview of the extend of morphological variability in the existing collection. The genotypes belonging to a particular cluster and having desirable traits can be hybridized with the other promising lines from different clusters, which may facilitate the accumulation of favorable genes in the progeny. The obtained hybrids may be fixed in the advanced generations, which may lead to development of high yielding varieties with desirable characteristics.

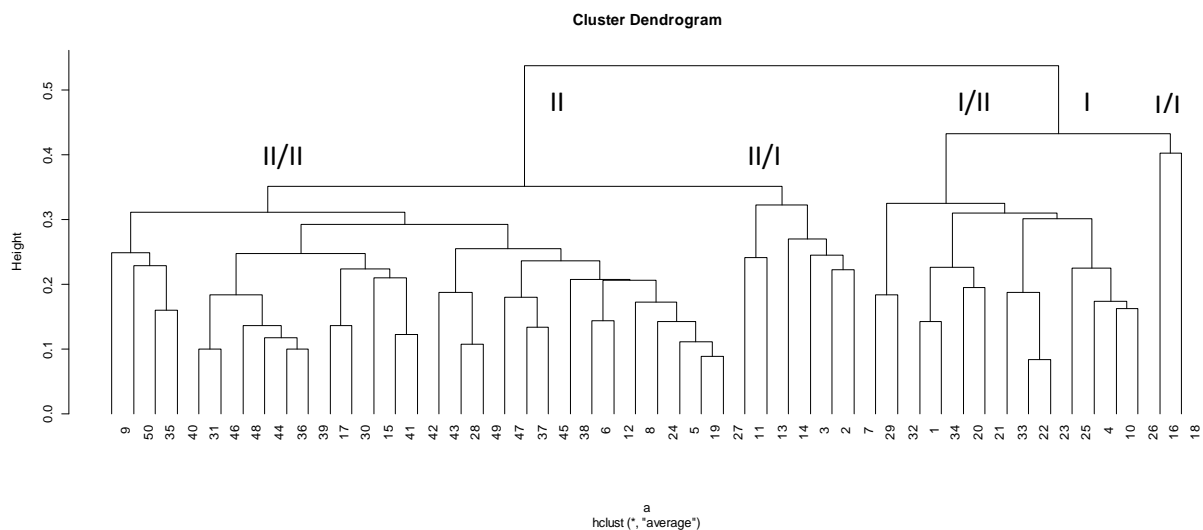


Figure 1. Dendrogram representing genetic distance between 50 opium poppy genotypes.

### Conclusions

The analyzed opium poppy genotypes showed high diversity for the morphological traits. Based on the morphological characteristics the germplasm lines were classified in two main clusters. Genotypes that had white petal color without blotch were located in cluster I and the genotypes with colored petals and different intensity of the violet color of blotch belonged to cluster II. All variants of other traits were present in both clusters. There was no association between genotype origin and the clustering pattern. The results of this study were useful for identification of the existing morphological variability in poppy collection. Further research is needed in order to evaluate different quantitative and qualitative traits and perform more precise classification.

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**THE PRODUCTIVE CHARACTERISTICS ON BLACK MAGIC TABLE GRAPE VARIETY, GROWING IN THE TIKVES'S VINEYARD, REPUBLIC OF MACEDONIA**

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**Abstract**

Table grape variety Black Magic was introduced in R. Macedonia in 2000. The variety is grown in the Tikveš vineyard area at 1.0 ha surface, on high cordon with trick system of irrigation. During the period of 2007-2009 Black Magic was studied including the following parameters: amount of harvested grape (total and packed), dimension and shape of cluster and berry, mechanical properties of berry (breaking resistance and resistance of pressure) and chemical content of must (content of sugar and total acids). On the base of obtained results, it was found that Black Magic variety had a high stability with no significant variation during the period of study. Thus, the average yield was 5.6 kg/vine, the cluster had average weight of 369 g and the berry 5.5 g. Furthermore, the berry had a high resistance of pressure (2.715 g) and high breaking resistance (811 g). In the period examined, the average content of sugar in the grape must was 126 g/L and total acids 5.0 g/L.

**Key words:** Black Magic, cluster, berry, mechanical properties, sugar, total acids

**Introduction**

In most of the vineyards in the Republic of Macedonia, particularly the Tikveš vineyard region with continental and partially Mediterranean climates, the agro-ecological conditions are favourable for successful cultivation of table grape varieties of all epochs of maturity. The Tikveš vineyard is characterized by daily mean air temperatures of 12.4 – 14.5°C with annual temperature sum of 4500 – 5300°C and vegetation temperature sum ranging from 3950°C to 4764°C. Total annual precipitation ranges from 440 – 740 mm, and from 250 – 310 mm during the vegetative growth cycle (Table 1).

Regular enrichment of the assortment, mainly with introduced varieties, has increased the offer both on the domestic and foreign markets in the last decade. The Black Magic variety is one of the many varieties that have been introduced so far (Victoria, clones of Italian grapevine varieties, Paglieri, Don Mariano). This variety was first obtained in Moldova by the Institute of Viticulture in Chisinau and was named Codreanca; it was rebranded as Black Magic in Italy, which is the international name as well.

The vine has medium vigour. It is an early variety (maturation period in mid July) with good cluster and berry shape. As the grapes reach its mature stage, berries detach pretty easily thus limiting the transportability (Marjone Bowen 2010). The clusters are medium sized, conical, occasionally winged shaped and loose with an average bunch weight of 450 – 500 g. The berry is medium sized to large with an average weight of 5.5 – 6 g. The berry is ovoid in

shape with dark blue coloration of the skin, neutral taste and 1 – 2 grape seeds per berry (Bozinovic 2010).

**Table 1 Climatic factors in Tikves vineyard**

Vegetative period	in days	229-239
Period of active vegetation	in days	207-229
Average annual air temperature	in C <sup>0</sup>	12.4 – 14.5
Sum of annual temperature	in C <sup>0</sup>	4500-5300
Sum of temperature in the vegetative period	in C <sup>0</sup>	3950-4767
Annual precipitation	in mm	440-740
Precipitation in the vegetative period	in mm	250-310

## Materials and methodes

### Materials

The studies of the Black Magic variety were carried out in the Tikveš vineyard at the production plants of AD Goce Del ev, Tikveš, Kavadarci. The plantation was established in 2002 with the use of certified planting stock produced in the Rauscedo reproduction centre, Italy. The vines is grown on high cordon with 1 m trellis distance and 2.3 m planting distance of the vines in the row. Total of 60 vines were analyzed, i.e., three repetitive analyses of 20 vines. The vines were optimally loaded with 20 buds per vine, distributed in two canes with 8 buds and 2 spurs with 2 buds. Regular agrotechnical and ampelotechnical measures were applied: soil cultivation, fertilization, irrigation, foliar feeding, pest and disease control, lateral shoot thinning, etc.

### Methods

The study was conducted in the period 2007-2009. Internationally recognized methods (O.I.V.) were used.

The yield was obtained by harvesting of the grapes of all the vines involved in the study, and the yield per vine and per unit area (ha) was calculated.

The dimension and shape of the cluster and berries were determined according to the CODE system issued by the International Organisation of Vine and Wine (O.I.V.).

The mechanical properties of the berry were evaluated by 2 elements: breaking resistance and resistance of pressure measured in grams per unit area (g/cm<sup>2</sup>) and the pedicel-berry detachment resistance in grams (g).

The chemical composition of the must is determined by measuring the content of sugar and total acids.

The content of the sugar was determined using the Exlo's device, and the total amount of acids was determined by volumetric method, using 0.025 mol/l solution of NaOH.

## Results and discussion

Table 2 shows the results of the amount of harvested grapes including the yield per vine and hectare (ha), and the amount of packed grapes expressed in percentage (%) and kilograms (kg). The yield ranges from 21400 kg/ha (2008) to 24300 kg/ha (2009). The years of study show insignificant variation of 6.87. The percentage of the packed grapes in the period of study ranges from 85% to 90%, with average of 88% for the same period.

This indicates that the Black Magic variety is characterized with high stability in both the quantity (total yield) and quality (% of packed grapes of extra quality) of the grapes.

**Table 2 Yield of grape**

Year	Total kg/vine	Packed kg/vine	%	Total kg/ha	Packed kg/ha	Refuze kg/ha
2007	5.5	5.0	90	24.009	21.735	2.174
2008	5.5	4.7	85	21.400	18.800	2.600
2009	5.6	5.0	89	24.300	21.692	2.608
<b>07/09</b>	<b>5.6</b>	<b>4.9</b>	<b>88</b>	<b>23.236</b>	<b>20.742</b>	<b>2.461</b>
CV%	1.04	3.63			8.11	

Table 3 shows the results of the weight and dimension of the cluster, and the number of fertilized berries. The cluster weight ranges from 353 g (2007) to 410 g (2009), average of 369 g for the study period. This indicates that the Black Magic variety belongs to large cluster varieties. The cluster length is 20.9 cm. The number of fertilized berries ranges from 62 (2009) to 82 (2008), and the coefficient of variation is 15.

**Table 3 The dimensions and shape of cluster**

Year	Weight (g)	Length (cm)	Width (cm)	L/W	Number of berries
2007	353	22.7	9.8	2.3	81
2008	344	17.6	9.7	1.8	82
2009	410	22.5	8.0	2.8	62
<b>07/09</b>	<b>369</b>	<b>20.9</b>	<b>9.2</b>	<b>2.3</b>	<b>75</b>
CV%	9.7	13.8	11.0	21.7	15.0

L/W: length/width

The values for the weight, dimensions and shape of the grains are shown in Table 4. Berries are with an average mass of 5.46 g, and 23.8 mm in length and 18.8 mm width. The form of the berry is one of the most stable features that distinguishes the varieties. It is determined by the values of the length/width ratio. In our study, the length/width ratio has an average value of 1.3, according to which they belong to the group of varieties with elongated elliptical shape (Bozinovic 2010). After years of testing, the values of the berry shape have been with minor variations (coefficient 4.5), suggesting that it is immutable biological trait of the varieties. The size of the berries of the table varieties is one of the characteristics that define the category of corresponding grape variety. According to the values of average diameter (length + width / 2), the Black Magic variety belongs to the group of varieties with large berries. All these features increase the market value of the variety.

**Table 4 The dimensions and shape of berry**

Year	Weight (g)	Lenth (mm)	Widht (mm)	L/W	Average diameter
2007	5.09	23.7	18.7	1.3	21.2
2008	4.85	21.2	18.6	1.2	19.9
2009	6.43	26.6	19.2	1.3	22.9
07/09	5.46	23.8	18.8	1.3	21.3
CV%	15.6	11.3	1.7	4.5	7.0

L/W: length/width

One of the very important properties of table varieties is the transportability which is determined by the pressure resistance of the berry and the pedicel-berry detachment resistance. The results of these surveys are given in Table 5. The berry pressure resistance ranges from 2414g/cm<sup>2</sup> (2007) to 2870 g/cm<sup>2</sup> (2008) or the average of 2715 g/cm<sup>2</sup> for the test period. The mean test period value of the pedicel-berry detachment resistance is 811g, ranging from 720 g (2009) to 880 g (2008). The results indicate that the Black Magic variety features high transportability which is primarily driven by the high resistance of the berry to pressure, while the pedicel-berry detachment resistance is significantly reduced by the sugar content increase (Figure 1).

**Table 5 The mechanical function of the berry**

Year	Resistance of pressure (g/cm <sup>2</sup> )	Breaking of resistance (g)
2007	2414	832
2008	2870	880
2009	2860	720
<b>07/09</b>	<b>2715</b>	<b>811</b>
CV%	9.6	10.1

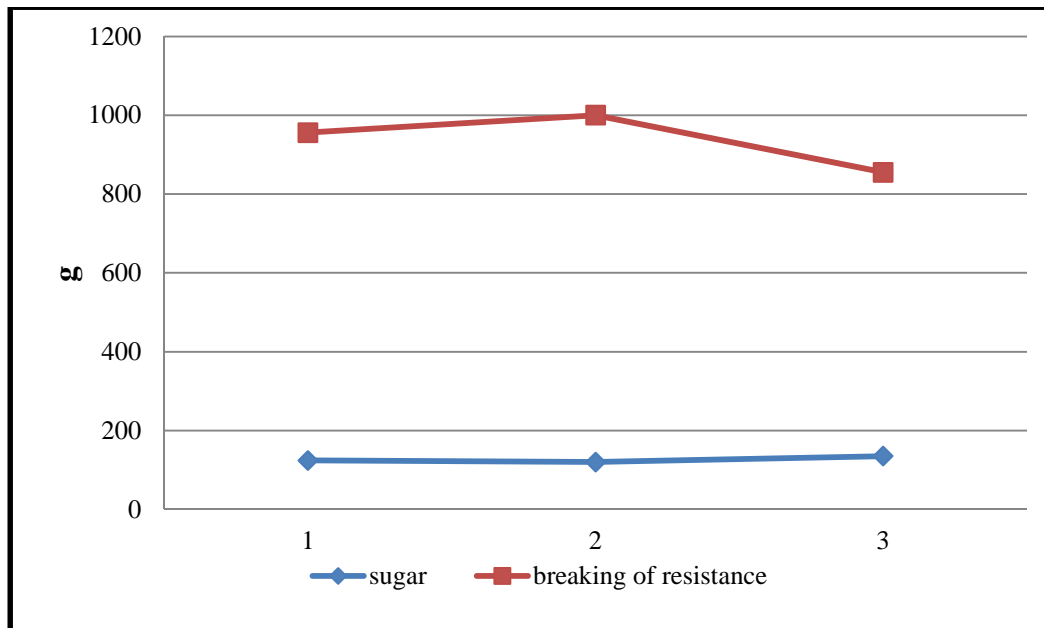
The results for the content of sugar and total acids in the must are shown in Table 6. The average sugar content of the test period is 126 g/l, and after years of testing it has been quite stable with a coefficient of variation of 6.1. The must sugar content ranges from 4.7 g/l (2009) to 5.2 g/l (2008) or 5.0 g/l average for the test period.

The content of sugar and total acid ratio determines the index of the maturity of the berries which primarily depends on the variety and also of the conditions of cultivation and application of agrotechnical and ampelotechnical measures during vegetation. Because of the balanced proportion between the sugar content and total acids, the grapes has pleasant and refreshing taste.

**Table 6. The content of sugar and total acids in the must**

Year	Sugar (g/L)	Total acids (g/L)	Index of maturity
2007	124	5.1	2.48
2008	120	5.2	2.31
2009	135	4.7	2.87
<b>07/09</b>	<b>126</b>	<b>5.0</b>	<b>2.52</b>
CV%	6.1	5.3	





1: 124 g/L, 2: 120 g/L and 3: 135 g/L

**Graph1. Breaking of resistance depending on the degree of maturity (content of sugar)**

### Summary

1. The Black Magic variety belongs to the group of high-yielding varieties. Its cluster is large and beautiful and it ripens early (I stage).
2. The balanced proportion between the sugar and total acid content gives the grapes a pleasant and refreshing taste.
3. Ampelotechnical measures (bunch tipping of the cluster and application of bio-stimulators) and special care during harvesting should be applied due to the low breaking of resistance of the berry.

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## INFLUENCE OF CROP SEQUENCE AND WEEDS ON MAIZE HEIGHT AND GRAIN YIELD

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### Abstract

The effect of two crop sequences: maize monoculture (MM), as old cropping system and maize-soybean-wheat crop sequence (MSW), as newer cropping system, was examined on plant height and maize grain yield. Both sequences had weed treatments: weed removal (B1) and weedy check (B2). Experiment was set up 2009 on experimental field of Maize Research Institute on calcareous chernozem soil type. After finishing first crop sequence (maize, soybean and wheat) maize height and grain yield were compared in monoculture and maize-soybean-wheat crop sequence.

In 2009, plant height and achieved grain yield had equal values in all treatments, as it was expected. In 2012, plants were higher in three crop rotation, for 16.3 cm (B1) and 23.6 cm (B2), then in monoculture. Maize grain yield was also higher in three crop rotation than in monoculture: in weedy check (B2) grain yield was higher 1.53 t/ha and in treatment with weed removal (B1) 1.49 t/ha. Based on LSD test, all these differences were significant except difference in yield between monoculture (MM) and three crop rotation (MSW) in treatment with weed removal (B1).

With respect to obtained results, it can be concluded that three crop rotation affected much more maize height and grain yield then it was present in monoculture.

**Key words:** maize, monoculture, three crop rotation, weeds and yield

### Introduction

Maize is a major crop in Serbia cultivated on the greatest arable area. Due to it, maize is partially grown in short- and long-term continuous cropping (Jovanovi , 1995), although it is not recommendable. In recent years, due to the occurrence of western corn root worm (*Diabrotica virgifera virgifera* Le Conte) that can cause great damages, avoidance of succeeding growing of maize on the same area has been recommended. This problem is easily solved by the application of crop rotation (Ba a et al., 2006 cit Stefanovi et al., 2011). Today, the crop sequence is one of important cropping practices, due to which optimum maize yields can be achieved without adverse effects on environments (Pop et al., 2009). In Serbia, maize is often grown in the rotation with other crops and this is well known "Balkan crop rotation system" in which maize and winter wheat rotate (Stefanovi et al., 2011). The crop sequence, as an element of the crop rotation, is alternation of two crops (Kova evi , 2003). Various effects on crops can be achieved by the application of the certain growing system. Since more intensive cultivation of legumes, first of all of soya bean, was initiated, a new type of crop sequence (maize-soya bean-wheat) has been applied. The presence of plants belonging to the family *Fabaceae* significantly contributes to the efficiency of the crop rotation, because the maize grain yield has been increasing, while the application of nitrogen fertilisers has been decreasing (up to 50%), which is a significant saving, and is important for

soil conservation (Videnovi et al., 2007). Therefore, long-term crop rotations that include legumes are much more agronomical acceptable than the short-term crop rotations (Stranger and Lauer, 2008).

The application of the crop sequence, and therefore the crop rotation, has positive effects on physical, chemical and biological properties of soil (Douglas et al., 2006; Aziz et al., 2011). Moreover, soil is less compacted (Šeremeši et al., 2008), and weed infestation can be reduced (Milošević et al., 2009; Dolijanović et al., 2011). Weeds, due to their great adjustability, can become resistant to certain cropping practice (Simić and Stefanović, 2008), but not to the crop rotation.

Yield is one of the most important reasons for the application the crop rotation rather than continuous cropping. Based on their long-term trials, Varvel et al. (2000) proved that the application of the crop rotation resulted in a lesser variation in yields. Not only does the crop rotation increase grain yield, but it can affect chemical composition of grain. In trials with the multispecies crop rotation (maize-soya bean-wheat-alfalfa) Riedell et al. (2009) established the increase in Na, Ca and Mg contents in maize shoots as well as in contents of N, S and oil in maize kernels. Therefore, the trial was set up with the aim to observe the effect of the maize-soya bean-wheat sequence and maize continuous cropping on the plant height and the grain yield of maize.

### Materials and Methods

The split-plot trial was set up in the experimental field of the Maize Research Institute, Zemun Polje in 2009. The following two crop sequence types were applied: maize continuous cropping (MM) and maize-soya bean-wheat sequence (MSW). The maize hybrid ZP 606 was sown in 2009 and 2012. Within both crop sequence types there were sub-treatments with (B2) and without (B1) weed presence, with four replications.

The plant heights were measured when maize pollination was over. Heights of 10 plants were measured and the average was calculated. Maize was harvested at the full maturity stage, and obtained weight was computed to 14% moisture.

Meteorological conditions in the two years of investigation (2009 and 2012) significantly differed in the sum and distribution of precipitation and average monthly air temperatures.

Table 1. Precipitation sums and average monthly air temperatures in Zemun Polje from March to October in 2009 and 2012

Months	Precipitation sums (mm)		Average temperatures (°C)	
	2009	2012	2009	2012
March	63.5	2.8	8.6	10.1
April	5.6	66.7	16.2	14.5
May	35.0	127.5	19.8	17.9
Jun	153.0	13.9	21.2	24.6
July	79.6	39.4	24.1	27.1
August	44.8	4.0	24.1	26.2
September	4.6	31.4	21.1	22.1
October	101.8	52.5	13.9	15.4
Sum/Average (March-October)	487.9	338.2	18.6	19.7

Unlike 2009 with a high precipitation sum well distributed over summer months (June, July and August) when maize plants have greatest needs for water, the corresponding period in 2012 was extremely dry (Table 1). Air temperatures also varied in these two years. Not only

did 2012 lack precipitation, but it also had high air temperatures that were higher by 2-3 °C in summer months than in 2009. Therefore, maize cultivation conditions in 2012 due to extremely dry and hot summer were very aggravated in comparison to 2009.

### Results and Discussion

Little work has been done to study effects of growing systems on the maize plant height. Generally, the plant height depends on the sowing density and rates of applied nitrogen fertilisers. According to results obtained by Modarres et al. (1998) and Hassan et al. (2000) the greater sowing density was the greater plant height, internode length and the ear height were. The plant height and the biomass yield increase up to the sowing density of 71,900 plants/ha and 280 kg N/ha, but any further increase in both sowing densities and nitrogen rates will not have any significant effect on the increase of the plant height and the biomass yield (Turgut, 2000).

The weather conditions during the performance of the experiment also affected the plant height, and therefore the grain yield of maize. Due to the sufficient precipitation sum and the favourable distribution of precipitation during the maize growing season in 2009, the plant heights were higher than the heights recorded in 2012.

However, what is important is that at the beginning of the study (2009) there was no difference in plant heights between maize continuous cropping and the maize-soya bean-wheat sequence (Table 2). As soon as the first maize-soya bean-wheat rotation ended, the significant difference between maize continuous cropping and maize-soya bean-wheat sequence was observed.

Table 2. Plant height in maize continuous cropping and maize-soya bean-wheat sequence with and without presence of weeds in 2009 and 2012

Replication	2009				2012			
	Weed removal B1		Control B2		Weed removal B1		Control B2	
	MM	MSW	MM	MSW	MM	MSW	MM	MSW
<b>I</b>	266.0	258.0	223.0	231.0	180.7	196.4	134.7	151.2
<b>II</b>	248.0	263.0	187.0	186.0	186.3	201.3	107.2	136.7
<b>III</b>	248.5	250.0	233.0	228.0	186.7	207.2	113.0	150.1
<b>IV</b>	259.0	247.0	216.5	212.0	190.9	205.2	120.0	131.2
<b>Average</b>	<b>255.4</b>	<b>254.5</b>	<b>214.9</b>	<b>214.3</b>	<b>186.2</b>	<b>202.5</b>	<b>118.7</b>	<b>142.3</b>
	<b>Crop seq. (A)</b>	<b>Weed treat. (B)</b>	<b>AxB</b>		<b>B1</b>		<b>B2</b>	
<b>LSD<sub>0.05</sub></b>	<b>48.04</b>	<b>40.11</b>	<b>41.19</b>		<b>6.51</b>		<b>16.24</b>	

Plants in the variant without weed presence (B1) were higher by 16.3 cm in the maize-soya bean-wheat sequence than plants in maize continuous cropping, while plants in the variant with weed presence (B2) were higher, on average, by 23.6 cm. The application of the LSD test showed that the differences between maize continuous cropping and the maize-soya bean-wheat sequence were significant. Gained results are in accordance with results obtained by Boomsma et al. (2006), who also recorded higher plants in the crop sequence than in maize continuous cropping.

Due to unfavourable weather conditions in the maize growing season in 2012, maize yields obtained in 2012 were lower than yields achieved in 2009. Furthermore, due to extreme weather conditions in 2012, maize grain yields recorded in maize continuous cropping differed from yields achieved in the maize-soya bean-wheat sequence (Table 3.)

Table 3. Maize grain yields in maize continuous cropping and maize-soya bean-wheat sequence with and without presence of weeds in 2009 and 2012

Replication	2009				2012			
	Weed removal B1		Control B2		Weed removal B1		Control B2	
	MM	MSW	MM	MSW	MM	MSW	MM	MSW
<b>I</b>	9.25	16.19	14.14	13.43	5.25	6.50	2.57	3.57
<b>II</b>	15.60	14.28	12.88	10.72	5.84	6.21	2.03	4.11
<b>III</b>	15.04	14.18	12.31	12.67	5.34	7.65	2.83	4.60
<b>IV</b>	14.71	14.92	12.77	12.46	5.27	7.48	3.21	4.31
<b>Average</b>	<b>13.65</b>	<b>14.89</b>	<b>13.03</b>	<b>12.32</b>	<b>5.43</b>	<b>6.96</b>	<b>2.66</b>	<b>4.15</b>
	<b>Crop seq. (A)</b>	<b>Weed treat. (B)</b>	<b>AxB</b>		<b>B1</b>		<b>B2</b>	
<b>LSD<sub>0.05</sub></b>	<b>4.79</b>	<b>4.67</b>	<b>4.81</b>		<b>1.59</b>		<b>0.77</b>	

Maize yields were, on average, higher by 1.53 t/ha and 1.49 t/ha in the maize-soya bean-wheat sequence in the treatment without (B1) and with (B2) weed presences, respectively, than yields obtained in maize continuous cropping. When the difference in grain yields is expressed in relative values, yields obtained in the maize-soya bean-wheat sequence are higher by 28.17 % (without weed presence) and 56.2 % (under conditions of weed infestation) than yields recorded in maize continuous cropping. Based on the LSD test, the difference obtained between maize continuous cropping and the maize-soya bean-wheat sequence was significant in the variant with weed presence, while this difference was not significant in the variant without weed presence.

Ciontu et al. (2011) also compared effects of maize continuous cropping and three-crop rotation (maize-soya bean-wheat) on maize grain yields and recorded yields in the three crop rotation higher by 830-1100 kg/ha (16.7-34.9 %) than in maize continuous cropping. When maize had been grown in the crop sequence, grain yields were higher by 10-17 % than yields of maize grown in continuous cropping (Higgs et al., 1990).

The higher plant height was the higher grain yield of maize was. According to this, it can be considered that there are a positive relationship between the plant height and the grain yield of maize. Golam et al. (2011) revealed that beside the ear height, the plant height of maize is exceptionally strongly positively correlated with the grain yield. Similar results obtained by Liu and Wiatracs (2011) indicated that there was a possibility to predict the yield on the basis of the plant height.

### Conclusion

The effect of the maize-soya bean-wheat sequence just after one rotation on the plant height and the grain yield is significant in comparison to maize grown in continuous cropping. It is important to emphasise that the difference in yields achieved in maize continuous cropping and the three-crop rotation (maize, soya bean and wheat) was significantly greater under more extreme conditions with weed presence than when weed was removed. Therefore, it can be stated that under poorer conditions maize should be rotated with other crops and should not be grown in continuous cropping.

The crop sequence and therefore the crop rotation express their actual effect after a few completed rotations and several years of application, hence it is necessary to continue these studies.

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## EFFECTS OF FERTILISING SYSTEMS ON MAIZE PRODUCTION IN LONG-TERM MONOCULTURE

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### Abstract

Maize monoculture is still present in Serbia and usually increases weed and pest infestation and decreases yield. It can also deteriorate physico-chemical and biological properties of soil. The objective of the study was to evaluate effects of an applied fertiliser system in long-term maize monoculture on soil properties and yield.

The experiment was set up at the Maize Research Institute (MRI), Zemun Polje, in 1972 and since then, the late maturing maize hybrid ZPSC 684 Ultra was continuously grown in the same field with the application of cattle manure each fifth year. The crop residues were ploughed down in the whole and half of the amount, and they were removed from the third variant. The mineral fertiliser application included three variants: without mineral fertilisers, the application of NPK fertiliser in autumn and of N in spring, and the application of only N fertiliser in spring. The content of N, P and K and organic matter in the soil was evaluated twice per maize growing season - at the beginning and at the harvest time, during the last two seasons - 2011 and 2012. The maize grain yield was evaluated and calculated at 14% moisture.

Crop residues increased the N, P, K and organic matter contents in the soil especially in the variant without application of cattle manure. In both years, the highest average maize yield was achieved with the application of cattle manure, whole amount of crop residues, and N fertiliser distributed over the soil surface at the beginning of the growing period. Nevertheless, although the late maturing hybrid potential and fertiliser rates were high in the maize monoculture, the highest yield amounted to not more than 10.4 t/ha. This underlines the importance of all cropping practices (crop rotation, fertilization, and hybrid type), their interaction and integrated effects on maize production.

**Key words:** monoculture, maize, fertilisers, soil properties, yield quality

### Introduction

Yield stability is an important characteristic to be considered when estimating the value of a cropping system. Maize monoculture and another two cropping systems are present in Serbia: maize continuous cropping (15%), two crop rotation (maize-winter wheat - 60% and maize-soybean - 15%) and three crop rotation (maize-winter wheat-soybean - 5%) (Jovanovi et al., 2004; Kova evi et al., 2005).

Growing just one crop in the same field for many years has some disadvantages. Maize monoculture usually increases weed and pest infestation and decreases crop yield. Furthermore, monoculture can deteriorate physico-chemical and biological properties of soil. On the other hand, maize is one of the crops tolerant to continuous cropping (Todorovi and Boži , 1995). According to global market demands, maize is a very profitable crop. But,

intensive and high yielding crop production implies the application of the cropping practices that will show results during a longer period of time. Studies carried out by Stranger and Lauer (2008) show that extended rotations involving forage crops, reduce N inputs, increase maize yield and are more agronomically sustainable than short-term rotations or monoculture. Results from the long-lasting study conducted at Zemun Polje, showed that the 21-year average grain yield of maize was the lowest in monoculture ( $6.75 \text{ t ha}^{-1}$ ), (Videnovi et al., 2007). Maize cultivation in continuous cropping with the application of the conservation tillage system and with leaving crop residues on the soil surface can possibly result in reduction of soil erosion (Papendick and Elliot, cit. Bullock, 1992).

It is known that each crop utilises different amounts of nutrients from the soil. Additionally, the composition and abundance of microflora differ among various individual crops, which affect the scope of transformation of organic and mineral matters into forms available to plants. According to studies conducted at Zemun Polje, the fertiliser application was the most effective in maize monoculture when yield has been increased by  $0.76 \text{ t ha}^{-1}$  (12.4%) after the application of  $160 \text{ kg N ha}^{-1}$ , even though the chernozem as a naturally fertile soil does not require intensive fertiliser systems (Videnovi et al., 2007). Effects of monoculture on microbiological processes is deleterious, which is particularly pronounced on less fertile soils, but the activity of micro-organisms in chernozem and similar soils is high in rhizosphere despite maize monoculture (Kovačević, 2010). Adiku et al. (2009) state that the crop rotation and residue management practices can significantly affect maize performances. Based on a long-term experimental study, the aim was to establish advantages and disadvantages of maize growing in continuous cropping. Besides, the objective was to determine the effects of the applied fertiliser system in long-term monoculture on soil properties and maize yield.

### **Material and Methods**

The experiment was set up at the Maize Research Institute (MRI), Zemun Polje, in 1972 with the aim to evaluate effects of maize growing in monoculture on crop production and soil properties. The late maturing maize hybrid ZPSC 704 was continuously grown in the same field with the application of cattle manure each fifth year. In the last three years the recently developed late maturing hybrid ZPSC 684 Ultra was grown in the experimental field in order to suppress perennial grass weeds by applying cycloxydim. The soil type in the experimental field is slightly calcareous chernozem with 53% sand, 30% silt, 17% clay; with good fertility and 3.3% of organic matter and moderate drainage. The pH in water is 6.9 and the soil structure is silty clay loam.

The cattle manure application is the first factor in the experiment and there were two variants of fertilisation:  $1080 \text{ kg cattle manure ha}^{-1}$  applied in 2008 and without application of cattle manure. The crop residues management is the second factor with three levels: ploughing down the whole (CR1) and the half amount (CR2), and they were removed from the third part of the experimental plot (CR3). The mineral fertiliser application, as the third factor, included three variants: F1 - application of NPK fertiliser in autumn (N:P:K= 15:15:15,  $1709 \text{ kg ha}^{-1}$ ) and N in spring (UREA,  $320 \text{ kg ha}^{-1}$ ), F2 - the application of only N fertiliser in spring (UREA,  $237 \text{ kg ha}^{-1}$ ) and F3 - without mineral fertilisers.

The content of N, P and K and organic matter in the soil was evaluated twice per a growing season - at the beginning and at the harvest time, during the last two seasons - 2011 and 2012. The maize grain yield was recorded and calculated at 14% moisture. The yield data were processed by standard deviation (SD).

*Meteorological conditions*

Meteorological conditions during growing seasons were different in 2011 and 2012. The precipitation was higher in 2011 (273.3 mm) than in 2012 (210.6 mm), while the mean daily temperatures were 20.1 °C and 22.1 °C, respectively. The year of 2012 was extremely dry (June - 30.7 mm; August - 5.8 mm and September - 26.0 mm) and unfavourable for maize production. A dry spell during the June-September period in 2012 year was especially important since it coincided with maize pollination and yield formation, Figure 1.

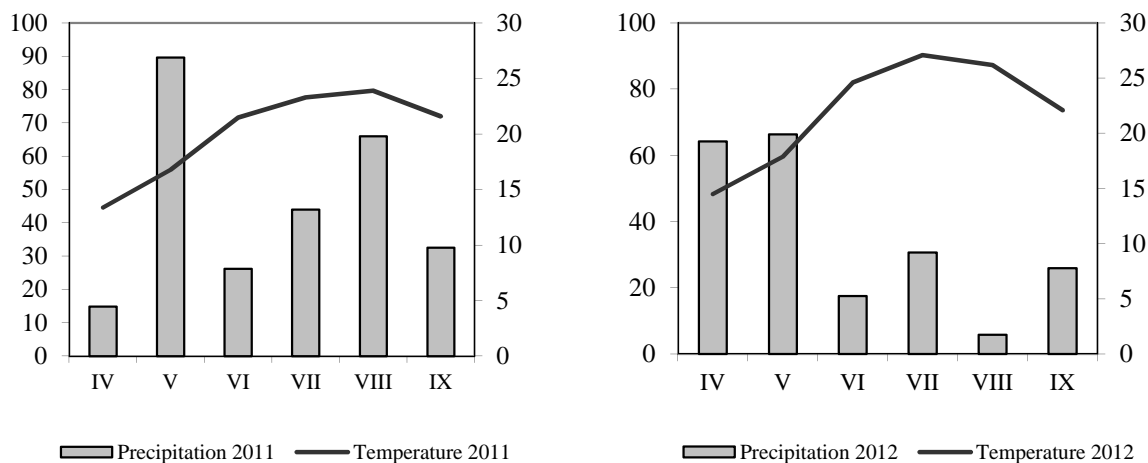


Figure 1. Meteorological conditions in 2011 and 2012

### Results and Discussion

Application of cattle manure and crop residues increased the N, P, K contents in the soil especially at the beginning of the 2011 growing season (Table 1). In the plot without application of cattle manure, ploughing down of the whole amount of residues contributed to the increase of the organic matter content even up to 0.5% (F3), while in the plot with the application of cattle manure the organic matter content did not depend on the return of crop residues. The content of analysed mineral nutrients and organic matter was also increased after the application of higher amounts of fertilisers (F1), in the variant with cattle manure.

The content of the available N was reduced until the end of the growing season, whether it is related to outtake with yields or losses. The highest N loss or the highest difference in N content in the soil between the beginning of the growing season and harvest, was recorded in F2 and CR2 in the variant without application of cattle manure ( $149.2 \text{ kg ha}^{-1}$ ). The highest P and K losses were in the variant F1, regardless of the level of crop residue returns. On the other hand, the application of cattle manure led to the increase of organic matter and nutrient contents in the soil, as well as to their greater outtake in all trial variants.

Table 1. Effects of different fertilising treatments on N, P, K (kg/ha) and organic matter (%) contents in the soil in long-term maize monoculture (2011)

		Beginning of growing season				Harvest			
		N	P	K	OM	N	P	K	OM
Without cattle manure									
Crop residues	F1	656.7	441.5	537.0	3.3	569.9	209.5	535.6	3.0
	F2	269.1	133.1	169.5	3.2	119.9	50.7	104.3	2.5
	F3	111.4	276.6	324.7	3.3	52.3	130.4	216.9	2.8
	<b>Av.</b>	<b>345.7</b>	<b>283.7</b>	<b>343.7</b>	<b>3.3</b>	<b>247.4</b>	<b>130.2</b>	<b>285.6</b>	<b>2.8</b>
½ of crop residues	F1	549.4	397.6	473.7	3.1	513.1	178.8	341.4	2.7
	F2	173.4	122.0	201.9	3.1	99.5	50.7	73.0	2.6
	F3	110.0	145.3	178.3	3.0	38.6	81.9	135.7	2.7
	<b>Av.</b>	<b>277.6</b>	<b>221.6</b>	<b>284.6</b>	<b>3.1</b>	<b>217.1</b>	<b>103.8</b>	<b>183.4</b>	<b>2.7</b>
Without crop residues	F1	417.9	299.0	531.3	2.8	308.2	157.0	276.7	2.6
	F2	161.0	78.9	124.2	2.9	97.6	58.6	70.4	2.5
	F3	61.8	153.5	209.8	2.6	28.0	57.1	131.9	2.5
	<b>Av.</b>	<b>213.6</b>	<b>177.1</b>	<b>288.4</b>	<b>2.8</b>	<b>144.6</b>	<b>90.9</b>	<b>159.7</b>	<b>2.5</b>
Average	279.0	227.5	305.6	3.1	203.0	108.3	209.6	2.7	
Cattle manure									
Crop residues	F1	709.6	666.5	442.9	3.2	497.1	380.6	936.8	3.2
	F2	277.8	518.2	654.6	4.1	256.8	333.3	366.6	2.6
	F3	361.4	542.6	707.4	3.6	153.1	242.3	338.8	2.6
	<b>Av.</b>	<b>539.6</b>	<b>575.8</b>	<b>601.6</b>	<b>3.6</b>	<b>302.3</b>	<b>318.7</b>	<b>547.4</b>	<b>2.8</b>
½ of crop residues	F1	615.9	590.9	238.1	4.0	545.7	466.6	746.2	3.5
	F2	423.4	550.0	507.2	3.0	256.0	330.8	295.5	2.9
	F3	314.5	449.7	450.7	3.3	188.4	346.3	313.8	3.3
	<b>Av.</b>	<b>451.3</b>	<b>530.0</b>	<b>398.7</b>	<b>3.4</b>	<b>330.0</b>	<b>381.2</b>	<b>451.8</b>	<b>3.2</b>
Without crop residues	F1	603.5	459.9	557.0	3.7	418.7	418.0	510.4	3.4
	F2	413.5	576.9	679.1	4.4	241.5	425.8	463.7	3.0
	F3	223.4	332.0	258.0	5.4	126.0	118.2	139.6	2.8
	<b>Av.</b>	<b>413.5</b>	<b>456.3</b>	<b>498.0</b>	<b>4.5</b>	<b>262.1</b>	<b>320.7</b>	<b>371.2</b>	<b>3.1</b>
Average	468.1	520.7	499.4	3.8	298.1	340.2	456.8	3.0	

Alongside stated trends, maize yield also varied (Figure 2). Cattle manure increased the maize average yield to the greatest extent (871 kg ha<sup>-1</sup>) in the variant in which the whole amount of crop residues was ploughed down and in the variant without application of mineral fertilisers.

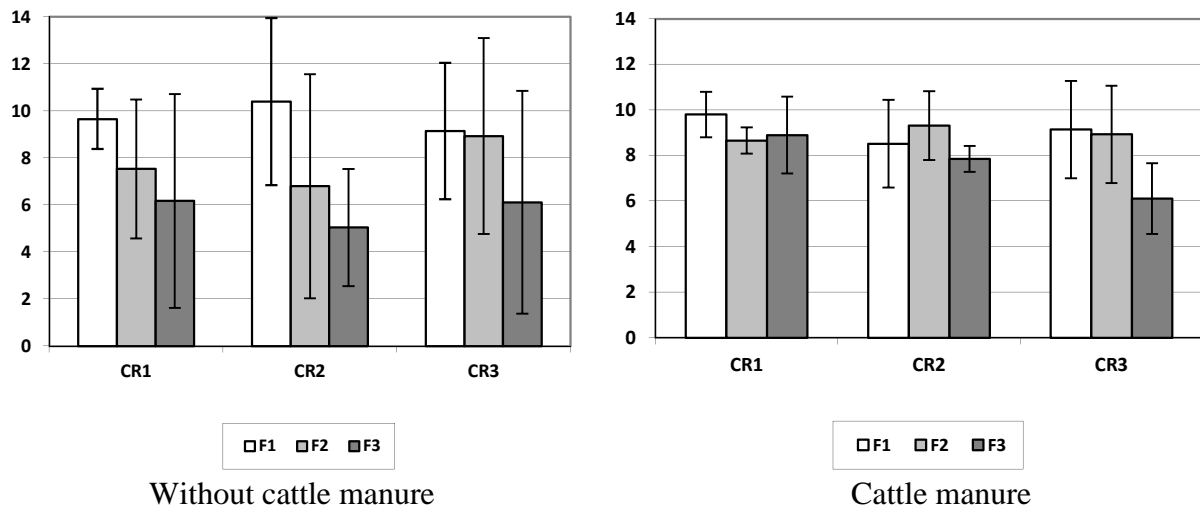


Figure 2. Maize grain yield (t ha<sup>-1</sup>) in dependence on crop residue management (CR) and the level of fertilising (F) in 2011 (mean value ± SD)

The statistical analysis of data indicates that the yield variation in 2011 in dependence on the application of mineral fertilisers and the return of crop residues was much less pronounced in the area in which cattle manure was applied. However, the highest yield was achieved in the variant without cattle manure with the return of a half of crop residues and with the application of NPK (CR2-F2- 10.4 t ha<sup>-1</sup>).

In unfavourable year of 2012, the macroelements and the organic matter content exchange was less intensive than in previous year because of extremely dry conditions. The N content was reduced with the maize yields and losses. According to analysis, the N content in the soil was lower on average by 57.7 kg ha<sup>-1</sup> at harvest (157.2 kg ha<sup>-1</sup>) than in the beginning of the growing season (214.9 kg ha<sup>-1</sup>) in the part of the plot in which cattle manure was not incorporated). Similarly, in the part of the plot in which cattle manure was applied, the N amount in the soil was reduced by 95.4 kg ha<sup>-1</sup> (Table 2). The outtake of P and K was significantly lower: 30.7 and 21.3 kg ha<sup>-1</sup>, respectively, in the part of the plot in which cattle manure was applied.

Table 2. Effects of different fertilising treatments on N, P, K (kg/ha) and organic matter (%) contents in the soil in long-term maize monoculture (2012)

		Beginning of growing season				Harvest			
		N	P	K	OM	N	P	K	OM
		Without cattle manure							
Crop residues	F1	178.9	170.6	56.8	2.9	127.9	154.0	39.6	4.7
	F2	285.3	84.7	26.1	3.2	219.0	80.8	22.4	5.0
	F3	112.8	48.7	24.4	3.0	94.2	26.7	17.9	5.0
	<b>Av.</b>	<b>192.3</b>	<b>101.4</b>	<b>35.8</b>	<b>3.1</b>	<b>147.0</b>	<b>87.1</b>	<b>26.6</b>	<b>4.9</b>
½ of crop residues	F1	201.4	367.4	131.7	3.6	203.4	223.9	51.1	4.8
	F2	376.7	97.1	57.1	3.5	291.8	49.3	17.2	4.5
	F3	176.7	47.1	28.9	4.1	173.3	41.8	11.3	6.6
	<b>Av.</b>	<b>251.6</b>	<b>170.5</b>	<b>72.6</b>	<b>3.7</b>	<b>222.9</b>	<b>105.0</b>	<b>26.5</b>	<b>5.3</b>
Without crop	F1	144.9	213.4	59.8	3.4	147.2	203.0	55.5	5.1
	F2	382.6	66.6	21.0	3.8	96.8	57.9	9.6	5.2

residues	F3	74.9	49.3	25.4	3.1	60.9	31.2	15.3	4.6
	<b>Av.</b>	<b>200.8</b>	<b>109.7</b>	<b>35.4</b>	<b>3.4</b>	<b>101.7</b>	<b>97.4</b>	<b>26.8</b>	<b>4.9</b>
Average		214.9	127.2	47.9	3.4	157.2	96.5	26.6	5.0
Cattle manure									
Crop residues	F1	372.1	314.7	82.9	3.1	155.4	312.1	50.6	4.3
	F2	380.3	291.6	53.7	3.6	352.6	194.0	18.5	4.4
	F3	308.3	234.8	52.1	3.6	147.6	142.0	34.5	4.7
	<b>Av.</b>	<b>353.6</b>	<b>280.4</b>	<b>62.9</b>	<b>3.4</b>	<b>218.5</b>	<b>216.0</b>	<b>34.5</b>	<b>4.5</b>
½ of crop residues	F1	314.9	318.2	63.4	3.6	266.3	335.5	35.4	4.8
	F2	436.8	233.1	33.9	4.0	378.0	194.0	25.6	7.5
	F3	259.3	189.4	32.4	2.8	208.8	196.6	19.9	4.5
	<b>Av.</b>	<b>337.0</b>	<b>246.9</b>	<b>43.2</b>	<b>3.5</b>	<b>284.4</b>	<b>242.1</b>	<b>27.0</b>	<b>5.6</b>
Without crop residues	F1	315.8	424.1	73.4	4.2	188.4	365.0	36.0	4.5
	F2	308.6	159.5	30.5	4.3	278.6	197.0	20.2	5.8
	F3	296.0	196.9	34.4	4.6	157.9	189.8	22.8	5.2
	<b>Av.</b>	<b>306.8</b>	<b>260.2</b>	<b>46.1</b>	<b>4.4</b>	<b>208.3</b>	<b>250.6</b>	<b>26.3</b>	<b>5.2</b>
Average		332.5	262.5	50.7	3.8	237.1	236.2	29.3	5.1

Unlike analysed macroelements, the content of organic matter increased to a smaller extent in all experimental variants and treatments during the growing season, probably as a result of the activation of soil microorganisms after rainfalls that occurred at the end of the growing season.

In 2012, under conditions of extreme drought, the application of cattle manure contributed to slightly higher maize yields (Figure 3). Regardless of the fact that stress conditions (high temperatures and lack of precipitation) prevailed during the growing season, the highest yield of over 7 t ha<sup>-1</sup> was obtained in the treatment CR2-F2 due to good nitrogen supply. This means that the optimum nitrogen supply with a prolonged effect of smaller amounts of ploughed down crop residues could provide substantial yields in maize continuous cropping (Kova evi , 2010). The statistical analysis showed that the yield recorded in dry 2012 similarly varied in dependence on applied cropping practices in the variant with and without application of cattle manure.

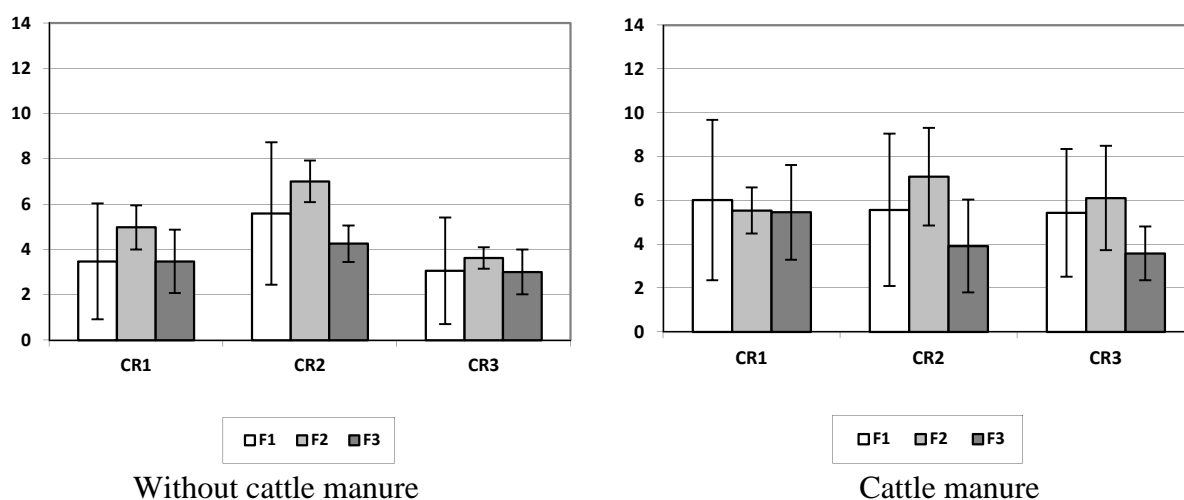


Figure 3. Maize grain yield (t ha<sup>-1</sup>) in dependence on crop residue management (CR) and level of fertilizing (F) in 2012 (mean value ± SD)

The presented results indicate that crop residues and amount of applied fertilisers, influence the rates of N, P, K and organic matter in the soil in maize monoculture. The present trend of increasing percentage of maize acreage emphasises the importance of developing improved procedures for achieving optimum N and other elements use. Generally, the basic information required in predicting the optimum use of N includes the internal N requirement by the crop for the expected yield, the amount of soil N mineralised during the cropping season, the amount of residual mineral N present in the root zone early in the cropping season, and the expected efficiency of recovery of the plant –available N supply (Stanford, 1973). At higher than optimum rates, a significant portion of the nitrate remains mobile and susceptible to loss by leaching or denitrification especially during optimally wet seasons.

In both years, the highest average maize yield was achieved with the application of cattle manure, whole amount of crop residues and N fertiliser distributed over the soil surface at the beginning of the growing season. The maize grain yield was significantly higher in both fertilising treatments (F1 and F2 – 9.44 and 8.36 t ha<sup>-1</sup>, respectively) in comparison to control (F3 - 6.70 t ha<sup>-1</sup>) in 2011 and also in 2012 (4.86, 5.73 and 3.95 t ha<sup>-1</sup>, respectively). On the other hand, there are no significant differences among applied rates of fertilisers, which is a result of the effects of meteorological conditions. Unfavourable meteorological conditions during 2012 reduced effects of fertilisers on maize yield in favourable year, 2011, but obtained differences were not significant. According to models integrating rainfall, fertiliser N and P rates, soil N and P, previous crop and tillage system accounted for 51 % of maize yield variability. Soil organic matter was not included in the models, but the indirect effect on yield was detected as organic matter correlated with initial soil N levels (Alvarez and Grigera, 2005).

Average maize yields were not the highest in the treatment with the highest amount of fertilisers (F1). It means that the application of the great rates of mineral fertilisers under the Zemun Polje conditions on slightly calcareous soil is not economically justified (Videnovi et al., 2007).

Regardless of the late maturing hybrid potential and high levels of the fertiliser application in maize monoculture the highest yield was lower than 10.4 t ha<sup>-1</sup> (CR2-F1 in 2011). This underlines the importance of all cropping practices (crop rotation, fertilizing, and hybrid type), their interaction and integrated effects on maize production.

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**IMPACT OF *LAETIPORUS SULPHUREUS* (BULL. EX FR.) MURRILL ON  
DESTRUCTION OF OAK WOOD**

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**Abstract**

Samples for the research have been taken from the heart wood zone of a sound tree of *Quercus petraea* agg. from the area of East Serbia, from association *Quercetum montanum* (Cer. et Jov., 1953). In the periods of 2, 4 and 6 months, the samples have been exposed to the impact of the mycelia of the brown rot fungus on oak tree: *Laetiporus sulphureus* (Bull. ex Fr.) Murrill (Sulphur Polypore). In order to determine the effect of *L. sulphureus* on decrease of the properties of oak wood, the following has been investigated: wood mass loss and hardness (according to Brinell). It has been concluded that mass loss of *Q. petraea* agg. wood under the impact of the fungus *L. sulphureus* after for 2, 4 and 6 months have been 0.90, 1.66 and 4.29% respectively. The hardness of the wood of *Q. petraea* agg. under the impact of *L. sulphureus* after 2, 4 and 6 months decreased to 96.44, 91.74 and 41.00%. In addition to separately displayed comparative review of the loss of mechanical properties in relation to mass loss, depending upon the duration of the impact of *L. sulphureus*, based on the review, the respective percentages of hardness of wood of *Q. petraea* agg. have been determined for the recorded mass losses in the period after 2, 4 and 6 months.

**Key words:** *Laetiporus sulphureus*, hardness; wood properties

**Introduction**

Development of the wood processing industry is causing growing demand for high-quality wood raw materials. This calls for preservation and extension of wood durability, which is directly linked to preservation of physical, chemical, mechanical, aesthetic and other properties, according to Miric & Popovic (1993) and Karadzic (2006).

Basic structural constituents of wood (cellulose, hemicellulose and lignin) are distributed in different percentages in different species and parts of trees, as discussed by Markovic *et al* (2011). There is thus more cellulose in soft than hard species of trees, in bolewood more than in branchwood, in early successional species more than in late ones, as discussed by Knezevic (1975) and Miric & Schmidt (1992). Moreover, the presence of cellulose determines the modulus of rupture, whereas the presence of lignin determines the bulk modulus and hardness (Miric *et al*, 2012). Through its enzyme system, the epixylous fungi break down the constituents of wood cell walls, modify the percentage of their participation and thus directly induce changes of the wood properties (Markovic *et al.*, 2011). The agents of brown prismatic rot (to which the researched fungus *L. sulphureus* belongs) disintegrate primarily cellulose, while the disintegration of lignin occurs in a far smaller extent. This paper presents the course of alteration (decrease) of the presence of lignin in the cell wall, reflected in the decrease of wood bulk modulus of *Q. petraea* agg. under the influence of the fungus *L. sulphureus* after 2, 4 and 6 months of the incubation.

## Material and method

The substrate used in the research was a 110-year-old healthy tree of Sessile oak *Q. petraea* agg. The tree had been cut in Eastern Serbia, at the altitude of 550 m in association *Quercetum montanum* (Tomic, 1992). As the fungus *L. sulphureus* attacks the first bole (technically the most valuable part of a tree), the analyses were conducted on a log 3.5 m in length (from the lower part of the trunk to the first live branch), which was according to the relevant pattern cut into specimens using the standard prescribed dimensions 2x2x4cm, in accordance with method by SRPS D.A1.040:1979.

Dikaryotic mycelia *L. sulphureus* was isolated from the sporocarp and resown into plastic Petri dishes containing malt-agar growing medium of standard concentration. The Petri dishes served as glass carriers in order to prevent excessive soaking of moisture from the growing medium, onto which wood specimens of Sessile oak were placed. On the top of the stack were Petri dishes with 5% water solution of boric acid intended to induce high relative air humidity, as the fungus *L. sulphureus* is known not to attack a tree under 20% of moisture, according to Karadzic (2010).

The dishes were kept in a closed sterilized chamber in the total darkness, and the temperature was controlled by thermograph throughout the experiment. Over most of the duration of the research the temperature in the chamber was at about 20°C, with brief time intervals at about 28°C, which are roughly optimal temperatures for the development of the researched species, in accordance with method by SRPS D.A1.058.

All data obtained were processed by applying the standard statistical methods; destruction results were compared using the single factor analysis of variance and the least significant difference test for the control group and the duration of the fungus impact (2, 4 and 6 months).

## Results and discussion

**Effect of *L. Sulphureus* on the wood mass loss** – Based on the analysis of the basic parameters presented in Table 1, it is evident that a great variability in data has occurred as a result of the non-homogenous structure of wood as a material and exposure to the influence of the fungus. The wood mass loss of *Q. petraea* agg. ranged from 0.76 to 1.02% in the sample group exposed to the influence of the fungus *L. sulphureus* for 2 months; from 1.07 to 3.49% in the sample group exposed for 4 months; and from 1.32 to 8.80% in the sample group exposed for 6 months. The values of the variation coefficient also demonstrate the existence of data variability; the variation coefficient is very high, particularly after 4 months - 41.68 and after 6 months - 57.54.

Table 1. Wood Mass Loss (%) under the Influence of Fungus *L. sulphureus*


	0 months	2 months	4 months	6 months
Number of measurements	30	30	30	30
Minimum amount	0.0	0.76	1.07	1.32
Maximum amount	0.0	1.02	3.49	8.80
Arithmetic mean	<b>0.0</b>	<b>0.90</b>	<b>1.66</b>	<b>4.29</b>
Standard deviation	0.0	0.07	0.69	2.47
Variation coefficient	0.0	7.83	41.68	57.54

Due to the evident dispersion of data, the percentages were calculated and differences thereof from the basic parameters, based on the arithmetic mean, i.e. average amounts; thus the

average wood mass loss *Q. petraea agg.* after 2 months of exposure to the influence of fungus *L. sulphureus* amounts to 0.90%, after 4 months of exposure to 1.66%, and after 6 months of exposure to 4.29%. This means that the greatest mass loss occurred between 4 and 6 months of exposure, when it increased by 2.63%. The mass loss in this period is by 0.97% larger than the total loss over the first four months of exposure to the fungus (1.66%), and three times as great as the loss occurred in the first 2 months (0.90%). According to the aforesaid, the most intensive mass loss occurred after 4 months although statistically significant differences, resulting from the influence of the fungus, were observed as early as within the first 2 months of exposure (Table 2).

Table 2. Wood Mass Loss (g) under the Influence of *L. sulphureus*

	0 months	2 months	4 months	6 months
0 months	-	-0.792667	-1.457	-3.92133
2 months		-	-0.66433	-3.12867
4 months			-	-2.46433
6 months				-

 - Significant difference at the level of 0.05

Based on the aforesaid, it may be concluded that the decomposition of wood under the influence of the fungus *L. sulphureus* commences in the first 2 months yet it is most intensive in the period from 4 to 6 months of exposure.

**Effect of *L. Sulphureus* on the wood hardness reduction (by brinell)** – It is well known that the hardness of healthy Sessile at the standard humidity of 12% amounts to minimum 42.0, 69.0 on the average and maximum 99.0 N/mm<sup>2</sup> (Soskic, 1994). Based on the results presented in Table 3, the hardness of the control sample group of *Q. petraea agg.*, equaled minimum 60.57, 80.10 on the average and maximum 104.5 N/mm<sup>2</sup>. The samples that had been exposed to the impact of the fungus *L. sulphureus* for 2 months exhibited average hardness of 77.25; after 4 months of exposure the average hardness was 73.48 and after 6 months - 32.84 N/mm<sup>2</sup>.

Table 3. Wood Hardness Reduction (N/mm<sup>2</sup>) under the Influence of *L. sulphureus*


	0 months	2 months	4 months	6 months
Number of measurements	30	30	30	30
Minimum amount	60.57	55.12	51.87	22.30
Maximum amount	104.50	104.50	96.33	89.08
Arithmetic mean	<b>80.10</b>	<b>77.25</b>	<b>73.48</b>	<b>32.84</b>
Standard deviation	9.95	14.14	11.29	13.36
Variation coefficient	12.42	18.69	15.37	40.68

From the results presented in Table 3, it is evident that the least data dispersion occurred in the control sample group, which is demonstrated by the variation coefficient amounting to 12.42. Slightly higher variability in data was observed after 4 months of exposure to the influence of the fungus *L. sulphureus* (with variation coefficient of 15.37) and after 2 months (18.69). extremely high variability in data was recorded after 6 months of exposure to the influence of the fungus (40.68). This indicates that in the period between 4 and 6 months of exposure, the wood hardness reduction under the influence of the researched fungus *L. sulphureus*, progresses rapidly and amounts to 32.84 N/mm<sup>2</sup> (probably due to uneven colonization of the wood, variation coefficient increases substantially).

Percentages and difference in the wood hardness of *Q. petraea agg.* under the influence of fungus *L. sulphureus*, depending on the time of exposure to the influence of the fungus, calculated as compared to the control sample group, demonstrate that the wood hardness reduction of *Q. petraea agg.* under the influence of fungus *L. sulphureus* in the first 4 months of exposure is rather weak (8.26%) in contrast to the reduction occurring between 4 and 6 months of exposure (50.74%), with the total wood hardness loss of 59.00% after 6 months. Significant wood hardness reduction in the period between 4 and 6 months of exposure to the influence of the fungus *L. sulphureus* is evident from the least significant difference test presented in Table 4.

Table 4. Wood Hardness Reduction (N/mm<sup>2</sup>) under the Influence of *L. sulphureus*

	0 months	2 months	4 months	6 months
0 months	-	2.85209	6.62043	47.2596
2 months		-	3.76834	44.4075
4 months			-	40.6391
6 months				-

 - Significant difference at the level of 0.05

Based on the results of T-test presented in Table 4, it is evident that all the respective differences between the 6 month-period and other groups compared are significant, with high amounts (differences range from 40.6391 to 47.2596), whereas the difference between the control group and 4-month period group is also significant but exhibits a far lower amount (6.62043), indicating that the wood hardness reduction commences in the period between 4 and 6 months of exposure to the fungus (differences between 0-month and 2-month groups as well as between 2-month and 4-month groups are random). Significant differences between the control group and 4-month group are only a result of the longer period of comparison between the two groups of data).

According to the literature, the wood hardness has proved to be the best indicator of the changes in the inner structure of wood. This is why by means of this property, destruction due to the impact of epixyloous fungi is most rapidly and most clearly observed (Jankovsky *et al.*, 2002). Given the fact that wood hardness depends on the quantity of lignin, it is clear that the researched fungus (although it causes brown rot, i.e. primarily disintegrates hemicellulose and cellulose), also disintegrates lignin in the period after 4 months of impact.

Table 5 presents wood hardness reduction in comparison to wood mass loss after 2, 4 and 6 months of exposure to the fungus impact. The loss of mechanical properties of wood is presented as the difference against the control group (100%).

Table 5. Differences in Property Decrease (%) under the Influence of the Fungus

Wood Property	Time of exposure to the fungus		
	2 months	4 months	6 months
Mass loss	0.90	1.66	4.29
Hardness by Brinell	3.56	8.26	59.00

Table 5 above shows the percentage of decrease in the tested mechanical property as compared to the mass loss after each respective incubation period.

**Correlation analysis of the wood properties and time of exposure to the fungus** - The correlation analysis was conducted in order to establish a correlation link between the tested properties of wood *Q. petraea* agg. depending on the time of exposure to the fungus (Table 6).

Table 6. Correlation analysis of Exposure time to Fungus and Wood Properties

Tested property	Model type	Correlation coefficient (r)	Regression equation
Mass loss ( $G_m$ )	Square function (y)	0.98582	$G_m = (0.094068 + 0.327671 \times T)^2$
Hardness ( $H_b$ )	Linear model	-0.84574	$H_b = 87.75 - 7.2775 \times T$

The performed correlation analysis of changes in mechanical properties of wood *Q. petraea* agg., depending on the time of exposure to the fungus *L. sulphureus*, has led to the conclusion that there is a strong correlation link between the variables. Under the influence of the fungus *L. sulphureus*, the correlation coefficient is the highest (indicating the strongest correlation link between the variables) with the mass loss (0.98582). The correlation coefficient under the influence of the fungus *L. sulphureus* is the lowest with the wood hardness (-0.84574), which is a consequence of a rapid decrease in wood hardness in the period from 4 to 6 months of exposure to the impact of the fungus. The presented regression equations open the possibility to forecast modifications in wood properties over certain time periods of exposure to the fungus, under unaltered environmental conditions. This is significant for practical purposes, i.e. for undertaking proper measures of protection, as well as from the standpoint of usability of wood.

### Conclusions

Based on the research presented, the following most relevant conclusions can be inferred: Wood mass loss of *Q. petraea*, under the influence of the fungus *L. sulphureus* after 2, 4 and 6 months of incubation amounted to 0.90%, 1.66% and 4.29%, respectively, which suggests that the greatest mass loss due to the impact of *L. sulphureus* occurs between 4 and 6 months. Wood hardness of *Q. petraea*, under the influence of the fungus *L. sulphureus* after 2, 4 and 6 months of incubation amounted to 96.44%, 91.74% and 41.00%, respectively, which suggests that the greatest wood hardness reduction due to the impact of *L. sulphureus* occurs between 4 and 6 months.

Correlation analysis showed a strong correlation link between the changes (decrease) in wood properties of *Q. petraea*. and the time of the influence of the fungus *L. sulphureus*. This opens the possibility to forecast modifications in wood properties depending on the time of exposure to the fungus under unaltered environmental conditions.

If a future research would carry out similar experiments on our most significant tree species against the greatest and most dangerous wood destructors, over a larger number of monitoring periods, the obtained results could serve as basis for creation of relevant tables (standards). By cross-referencing the obtained data and conducting their statistical analysis, we would arrive at the closest approximation of values to be inserted into relevant tables and applied in practice.

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**ADVANTAGES AND DISADVANTAGES  
OF THREE MATHEMATICAL MODELS OF A PEAR BORDER**

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**Abstract**

Various factors have influence on the growth and development of bio-materials. Consequently, shape variability is very important and should be examined. In many processes of heat exchange, as well as in other processes in bio-material handling, the physical properties of a fruit such as dimensions, shape, surface area and volume play significant role. The purpose of this study is to find a function which approximates a pear border line as precisely as possible. One type of estimation of an average pear border line was relying on the sixth order polynomial and proposed algorithm. Also, another two different ways of calculating the Williams pear border line were shown earlier. The first one included spline functions for an estimation of a pear border line, while the second way used regression function obtained by the nonlinear regression method. The regression function had two independent variables, the length and total length of a pear. Border lines of all pears in the sample were fitted with one regression function with large precision ( $R^2=97.48$ ). The surface area and volume of a pear were calculated based on the regression function and total pear length. In this paper, it is compared three different ways of pear border line calculation.

**Key words:** shape variability, integral calculus, cubic spline, nonlinear regression

**Introduction**

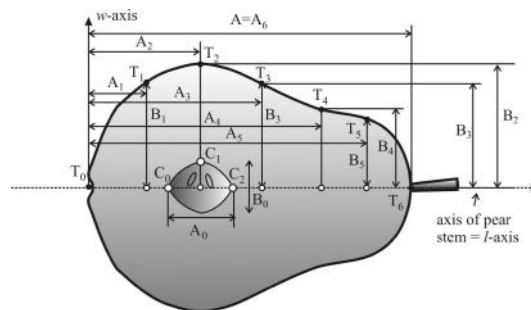
Physical properties of fruit (dimensions, shape, surface area and volume) have very important role in many processes of heat exchange and other processes of biomaterial handling (Mohsenin, 1980). It is well known that fruits, including pears, are dominantly irregular in shape. Certain number of measurements must be made for full characterization of fruit shape. The analysis of three mutually perpendicular axes usually contains enough information for the volume or surface area modeling. The finite element method was used to discretize the governing differential equations over the actual 3D pear geometry (Wang *et al.*, 2006). The pear dimensions were evaluated during the drying process and those data were used to calculate the pear surface area and volume. Cut pears were photographed horizontally and vertically against a millimeter-scaled paper. The shape of the whole pear was replaced with two regular bodies, half of a sphere and a cone (Guine *et al.*, 2006). The dimensions and volume were also investigated for cherries (Ochoa *et al.*, 2007), almond cultivars (Altuntas *et al.*, 2010) and mango (Spreer and Müller, 2010). If color is an important factor, then the use of digital images is essential (Quevedo *et al.*, 2009; Purlis and Salvadori, 2009; Altuntas *et al.*, 2010;). Xiao-bu *et al.* (2010) also used digital images to detect the apple defects. Optimization of digital images was widely studied (Acketa and Mati -Keki , 2000; Mati -Keki *et al.*, 1996). An image processing-based method is appropriate for measuring the volume and surface area of ellipsoidal agricultural products such as lemons, peaches, limes and eggs (Sabliov *et al.*, 2002).

Now, let us make a short introduction to the one of three methods, that are going to be presented here. For various kinds of approximation problems, it is frequently advantageous to use piecewise polynomials instead of polynomials because using low-degree polynomials locally is usually more accurate and more efficient than using a high-degree polynomial globally (Ascher *et al.*, 1995). The main property of cubic spline function is that it remains twice continuously differentiable over the observed interval. Program package Mathematica 6 (Wolfram, 1991) was employed for testing the cubic spline approximation of pear border line and for all necessary numerical integrations. This software is also very applicable in many other problems related to agriculture (Bodroža-Panti *et al.*, 2008) and to optimization (Mati -Keki and Acketa, 1997; Acketa *et al.*, 2000).

The purpose of this study was to compare various mathematical expressions for Williams pear border line. Those expressions allow easy estimation of the pear surface area and volume. Dedovi *et al.* (2011) confirmed the precision of mathematical model for the pear border line approximation using the following procedure: 1. volumes of the pears were measured by Archimedes' method; 2. volumes were calculated using numerical integral calculus with assumption that pear could be observed as a rotation body; 3. relative errors for calculated volumes were given.

### Material and Methods

Thirty fruits of Williams pear (*Pyrus communis*) were randomly selected and then halved through the longitudinal axis. Each half was split along the same axis to generate two pear quarters. The core and seeds were removed and the half of a pear was placed in a two-axis system, such that the total length ( $L$ ) of the pear was on  $l$ -axis, and the width ( $W$ ) was on the  $w$ -axis. Zero point was placed at the bulbous end of the pear (see Fig. 1).



**Figure 1.**<sup>1</sup> The pear outline and its measured points  $T_i (A_i, B_i)$ ,  $i=1, \dots, 6$ , as well as dimensions of pear core  $A_0$  and  $B_0$ . Point  $T_0(0,0)$  is start point of Cartesian coordinate system with  $l$ -axis (length) and  $w$ -axis (width);  $A_1=0.5 \cdot A_2$ ,  $A_3=0.5 \cdot (A_2+A_4)$ ,  $A_5=0.5 \cdot (A_4+A_6)$ ,  $C_0=(0.5 \cdot (A_2-A_0), 0)$ ,  $C_1=(A_2, B_0/2)$  and  $C_2=(0.5 \cdot (A_2+A_0), 0)$ .

The coordinates of the seven points, located on the pear border line  $T_i(A_i, B_i)$ ,  $i=0, \dots, 6$ , are presented in Babi *et al.* (2012) and Dedovi *et al.* (2011).

Among basic dimensions, thickness of the pears was not measured because it was assumed ([www.rainierfruit.com/products/pears/img/pears.pdf](http://www.rainierfruit.com/products/pears/img/pears.pdf)) that pear could be represented as rotating body, where “stem axis” was, actually, the axis of rotation. According to this assumption, pear thickness is equal to pear width, and the surface area and volume of the pear

<sup>1</sup> This figure is taken from Babi *et al.* (2012).



can be calculated using formulas (1) and (2), respectively. However, the pear border line function  $f(x)$  must be defined first. Generally speaking, the formulas for the surface area  $S$  and volume  $V$  of the rotating body within the interval  $(a,b)$ , where  $a$  is the starting point on the  $x$ -axis and  $b$  the last point on the same axis, for a non negative period of function  $f(x)$  are:

$$S(f(x),a,b) = 2 \int_a^b f(x) \cdot \sqrt{1+(f'(x))^2} dx \quad (\text{the surface area of rotating body}) \quad (1)$$

and

$$V(f(x),a,b) = \int_a^b (f(x))^2 dx \quad (\text{the volume of rotating body}) \quad (2)$$

The volumes of pears from the sample were measured by Archimedes' method and calculated by formula (2), where  $f(x)$  was appropriate border line function (polynomial function (4), spline function (5) or regression function (6)). Also, the surface area of pears from the sample, were calculated by formula (1), where  $f(x)$  was border line function.

The quarter of one pear was additionally bounded with two lateral flat surfaces. Flat surface area, denoted as  $FS$ , bounded by the  $x$ -axis and  $f(x)$ , was calculated as:

$$FS(f(x),a,b) = \int_a^b f(x) dx \quad (3)$$

for  $f(x) \geq 0, x \in (a,b)$ . The surface area and volume of a seed core, can also be calculated by using (1) and (2) since seed core can be considered as a rotating body, too.

## Results and Discussion

### 1. Polynomial fitting

In Babi *et al.* (2012),  $f(x)$  is the function which approximates the pear border line passing through the seven points  $T_i, i=0,1,2,\dots,6$  on the average pear border line. The function  $f(x)$  is, actually, sixth order polynomial  $P(l)$ ,

$$P(l) = 4.1135 \cdot l - 0.2531 \cdot l^2 + 0.0093 \cdot l^3 - 0.0002 \cdot l^4 + 2.083 \cdot 10^{-6} \cdot l^5 - 8.5968 \cdot 10^{-9} \cdot l^6 \quad (4)$$

where  $l \in [0, \bar{L}]$  is an independent variable, i.e.  $l$  is pear length which takes values from 0 to  $\bar{L}$ , while the average total length of pears is marked as  $\bar{L}=84.3$  mm.

### 2. Spline functions

The next objective is to determine the polynomial function of lower order than sixth, with the same outline representation. High order of polynomial  $P(l)$  produces very small coefficients which multiply  $l^5$  and  $l^6$ , causing the possible less precision and greater errors during the numerical calculations of the volumes and surfaces area. If cubic spline involves all seven characteristic points  $T_i, i=0,1,\dots,6$ , the interval  $[0,84.3]$  has to be split into four parts. The first subinterval  $[0,29]$  contains points  $T_0, T_1$  and  $T_2$ , the second one  $[29,44.5]$  contains  $T_2$  and  $T_3$  points, the third subinterval  $[44.5,60]$  contains points  $T_3$  and  $T_4$ , and the fourth subinterval  $[60,84.3]$  contains points  $T_4, T_5$  and  $T_6$ . Thus, cubic spline  $s(l)$  in (5) is represented by four 3rd order polynomial functions (one polynomial for each subinterval).

In the case when total length of a pear is  $L=84.3$ , the function describing pear border line is

$$s(l) = \begin{cases} 3.09764 \cdot l - 0.0984982 \cdot l^2 + 0.00102846 \cdot l^3, & l \in [0, 29], \\ 30.1456 - 0.011803 \cdot l + 0.00841199 \cdot l^2 - 0.000196814 \cdot l^3, & l \in [29, 44.5] \\ -75.7114 + 7.10387 \cdot l - 0.151026 \cdot l^2 + 0.000994001 \cdot l^3, & l \in [44.5, 60] \\ 628.3 - 27.9943 \cdot l + 0.432242 \cdot l^2 - 0.00223695 \cdot l^3, & l \in [60, 84.3] \end{cases} \quad ((5))$$

Two previously described methods were based on the same idea. Firstly, the functions which approximate average pear border line (polynomial or spline functions) pass through all seven points on the average pear outline. Secondly, those functions are later used for stretching or compressing of pear border line for each pear, using the previously proposed algorithm. However, there is the following limitation: each total pear length requires new polynomial or spline function to be created (meaning that each pear from the sample has a shape which can be obtained by stretching or compressing the average pear border line). In general, it cannot be correct because the shape of a pear from the same cultivar does not have to be equal to the shape of an average pear. These problems can be overcome by using the following approach.

### 3. Nonlinear regression

Dedovi *et al.* (2011) created only one function (6)

$$Q(l, L) = -\frac{40.6437}{L^5} \cdot l^6 + \frac{116.805}{L^4} \cdot l^5 - \frac{130.088}{L^3} \cdot l^4 + \frac{72.6492}{L^2} \cdot l^3 - \frac{22.9904}{L} \cdot l^2 + 4.26753 \cdot l \quad ((6))$$

which described the variability of pear boarder line. Very high coefficient of determination  $R^2=97.48\%$  was obtained for the confidence level of 95%. The main advantage of this model is that there is no need for extra algorithm given in Babi *et al.* (2012). Another advantage is that this (third) method is more precise than the first two methods previously described because all experimental points were used in nonlinear regression method and not only the mean values of seven coordinates on the pear border line.

## Conclusions

The Williams pear border line is approximated by: polynomial function, spline function and nonlinear regression function.

Cubic spline has similar properties as the polynomial function for the average pear border line approximation. The only advantage of cubic spline is more acceptable polynomial coefficients.

Pear border line variability implies that priority should be given to the nonlinear regression function for the following reasons:

it calculates pear border line for smaller and larger pears more precisely than other two proposed functions;

the volumes obtained by using the regression function as a pear border line approximation differs from the exact volumes (measured by Archimedes' method) with relative error 6.97%.

Removing the three smallest pears from the sample, relative error is then 4.24% only (Dedovi *et al.* 2011). Very high precision implies that surface area of a pear can be calculated very precisely as well.

it is clear that precise determination of the pear border line approximation, implies that calculation of the surface area and volume of a pear will be more accurate;

only one function is sufficient for pear border line formation, for arbitrary total pear length;

regression function does not require any additional algorithm.

These results are useful for packing, storage as well as in technology processes, biomaterial handling and drying processes.

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## GENETIC VARIATION OF MACRO AND MICROELEMENTS IN GRAIN OF MAIZE INBRED LINES

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### Abstract

In any breeding program, germplasm screening for a trait of interest is an important first step to genetic improvement. In the case of biofortification, nutritional breeding also starts with assembly of parental germplasm for crosses based on the evaluation of a large amount of genetic material. The objective of this work was studying the variability of macro and microelements in grain of 74 maize inbred lines. The highest protein content had lines from European germplasm and the lowest line of the Lancaster heterotic group. Lines from the BSSS group had the lowest oil content and the lines of Lancaster heterotic group the highest. Starch content was highest in lines from Lancaster heterotic group and lowest in the lines of European germplasm. The highest average concentration of Mg, Fe and P had inbred lines from European germplasm while inbred lines from BSSS germplasm had the highest Zn concentration. Lines from BSSS had the lowest average Mg and Fe content as well as lines from Lancaster germplasm had the lowest Zn and P content.

**Key words:** maize, macroelements, microelements, inbred lines

### Introduction

In past crop breeding and production have been focused on increasing yield and maintaining food and feed production stability. At the same time concentration of essential macro and micro nutrients have been decreased. From the nutritional point of view corn grain such as fat, protein, and starch content define corn quality characteristics. Chemical content of maize grain is very important for human and animal diets. Average content of proteins, oil and starch in maize seed is 9%, 4%, 73%, respectively (Balconi *et al.*, 2007). Micronutrients play important physiological roles in humans and animals (Cakmak, 2008, Fisher, 2008). While the traditional remedy of micronutrient deficiencies was in a form of food/feed supplements, suitability of agricultural strategies for enhancing micronutrient concentrations in grain was recently being assessed as a sustainable, long-term solution. Among these strategies, plant breeding strategy (biofortification) appears to be the most sustainable and cost-effective approach (Welch and Graham, 2002; Cakmak, 2008), especially for Fe and Zn. Quantities of micronutrients in maize grain are influenced by numerous complex factors including genotypes, soil properties, environmental conditions and nutrient interactions. Although results reported in literature about micronutrient concentrations in maize grain varied substantially, according White and Broadley (2005) considerable genetic variation in maize can be harnessed for sustainable biofortification strategies. Simic *et al.* (2009) studied relations among boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and

zinc (Zn) concentrations in grain of 297 genotypes of a maize population and suggested that it is possible to improve density of various micronutrients in maize grain simultaneously, although the progress would be very slow. The aim of this study was to investigate chemical composition of grain of 74 maize inbred, belonging to different heterotic groups, to define their nutritional quality.

### Material and methods

Grains from 74 maize inbred lines with different genetic background from MRI breeding program were used. The nine inbred lines belonging to European germplasm, 48 to BSSS, and 17 to Lancaster germplasm. Seed protein, oil and starch content were determined by near-infrared reflectance spectroscopy (NIRS) using Infratec 1241 Grain analyzer, (Foss Tecator, Sweden) and expressed in a percentage of absolutely dry matter of grain (ADM). Microelements were measured by Inductively Coupled Plasma - Optical Emission Spectrometry after wet digestion with HNO<sub>3</sub>+HClO<sub>4</sub>.

### Results and discussion

It is well known that total protein composition varies among genotypes (Mladenovic Drinic *et al.*, 2009, Stevanovic *et al.*, 2012). The total protein of 74 maize inbred lines ranged from 7.55% to 13.69%, with average value of 10.48% (Table 1). The oil content ranged from value of 2.87% to 5.06%, with average value of 3.95%. According to fact that starch is main constituent of maize grain (Balconi *et al.*, 2007), its content ranged from 67.29% to 73.16.8%, with average content of 69.91%. Inbred line that has the highest protein content has the lowest starch content. Obtained values for protein, oil and starch are typically for maize genotypes that were not specifically selected for those traits (Pollak and Scott, 2005; Harrelson *et al.*, 2008; Idikut *et al.*, 2009).

Table 1. Macro and micro content of maize inbred lines

line	proteins	oil	starch	Mg mg/g	Zn mg/g	Fe mg/g	P ug/g
BSSS							
2	8,31	3,20	72,13	965,94	17,84	25,18	7029,79
3	10,83	3,17	70,50	949,69	20,47	17,09	7239,35
6	9,05	<b>2,87</b>	71,31	897,19	22,12	23,65	8287,15
7	9,71	3,35	72,10	862,50	22,00	16,18	7677,52
8	8,86	3,07	70,26	774,69	20,28	21,00	7887,08
11	13,55	3,56	67,75	750,32	17,56	15,81	7144,09
13	8,10	3,42	72,18	647,82	27,031	5,90	6553,52
20	10,55	3,59	70,20	872,81	16,06	12,25	7620,37
24	11,51	4,25	69,97	785,31	25,15	22,75	7277,45
30	10,68	3,89	70,46	718,12	<b>34,59</b>	22,03	6839,28
31	10,09	3,35	71,42	842,50	27,09	23,28	7487,01
32	9,60	3,08	<b>73,16</b>	953,12	29,18	23,03	6934,53
33	10,98	3,60	70,32	718,75	21,18	5,59	7391,76
34	10,97	3,67	70,22	784,37	22,62	13,46	7182,20
36	10,22	3,87	70,00	889,37	20,90	11,59	7144,09
38	12,90	3,89	68,50	895,00	23,78	18,75	7544,16
39	8,89	3,21	71,75	696,25	17,31	<b>2,31</b>	7334,60

40	12,19	3,34	69,34	857,50	15,68	4,06	7810,88
41	10,00	3,85	71,10	693,75	34,12	4,81	6572,57
42	10,48	3,21	71,12	862,81	15,84	8,21	8020,44
43	12,93	3,43	68,15	811,87	16,12	15,62	7639,42
44	11,19	3,80	69,39	717,81	11,59	14,06	7201,25
50	10,45	3,74	70,10	780,94	15,68	11,84	6801,18
51	12,12	3,13	69,16	852,81	25,12	23,65	7105,99
53	9,07	4,20	70,70	633,12	15,78	16,62	5734,32
61	11,90	3,55	69,75	664,37	23,25	27,90	6439,21
62	10,10	3,29	71,10	614,37	12,31	10,59	5962,94
63	11,19	3,51	70,15	711,25	22,46	22,03	6153,44
64	10,64	3,55	69,38	<b>587,81</b>	30,93	18,12	6039,14
65	<b>13,69</b>	3,83	<b>67,29</b>	700,94	16,21	18,43	7125,04
66	11,67	3,20	69,19	827,81	<b>10,84</b>	20,093	8287,15
67	9,95	3,50	71,01	898,75	26,37	30,50	8020,44
68	10,55	3,77	68,66	930,31	32,25	24,59	8249,05
69	10,85	3,66	69,86	696,87	13,31	12,21	7125,04
71	10,31	3,51	70,80	626,25	30,31	21,18	6039,14
72	10,37	3,25	70,85	738,75	16,28	12,00	7658,47
73	10,08	3,06	71,76	753,75	11,96	10,12	7125,04
74	8,69	3,20	72,25	672,19	18,40	12,18	6686,879
75	7,64	3,75	70,95	762,81	14,68	10,34	7125,04
76	10,50	3,59	70,76	689,06	14,93	13,21	7201,25
77	12,62	3,30	69,18	620,31	17,25	16,4375	6934,53
4	8,97	3,89	70,80	827,18	20,12	21,59375	7696,57
5	9,58	4,58	71,12	725,94	18,96	25,46	6801,18
14	10,35	4,03	70,54	649,06	14,00	9,75	7372,70
27	10,82	3,69	69,46	<b>980,62</b>	35,75	24,15	7677,52
28	9,02	3,69	71,04	949,37	23,03	20,50	7906,13
29	<b>7,55</b>	3,27	71,96	835,94	26,53	21,40	6572,57
16	9,98	3,42	70,91	597,18	18,90	9,00	6020,09
average	10,38	3,54	70,42	776,56	20,92	16,47	7159,97
Lancaster							
9	10,49	4,19	67,23	836,56	24,34	20,43	8439,56
15	8,72	3,71	70,87	774,68	20,15	13,56	7315,55
17	10,11	4,53	69,02	775,00	15,59	28,96	7448,91
21	9,87	3,84	70,15	827,18	21,00	14,65	7582,26
26	8,20	3,75	69,45	887,50	27,18	26,09	7125,04
35	9,37	3,56	70,81	745,31	17,87	10,34	6915,48
45	11,06	3,61	69,65	783,43	16,59	19,37	8172,84
46	12,56	4,34	67,64	926,25	21,84	22,65	<b>8706,27</b>
47	10,23	4,22	70,04	867,18	18,00	13,43	7887,08
48	9,74	4,70	70,00	750,00	17,68	11,28	7525,11
49	11,86	4,07	69,39	655,94	15,40	12,90	<b>5315,20</b>
56	9,21	4,84	71,61	652,50	16,21	20,65	6058,19
57	8,48	4,72	69,13	682,50	13,03	18,59	6724,97
58	9,93	3,89	70,20	801,56	17,65	16,34	6648,77
59	8,38	3,62	70,74	751,25	18,65	15,50	6248,70

60	9,94	3,93	70,33	726,25	18,15	16,46	6267,75
55	11,45	4,50	69,13	797,18	22	12,71	7144,09
average	9,97	4,12	69,73	778,84	18,90	17,29	7148,58
European germplasm							
1	12,61	<b>5,06</b>	67,35	846,25	20,18	17,65	6686,87
10	11,61	4,32	69,05	894,37	21,96	18,96	7791,82
12	12,51	3,96	69,65	726,25	20,15	21,00	7201,25
19	10,56	3,51	68,99	803,75	15,18	27,56	7868,03
22	12,52	4,30	68,37	935,94	19,59	10,18	8611,01
23	10,12	4,06	71,27	711,56	22,28	19,00	6934,53
25	9,89	4,03	70,60	846,87	22,37	18,50	6991,69
54	10,07	4,87	69,28	722,81	20,50	23,62	7601,32
78	9,85	3,62	71,79	768,75	20,28	15,62	7868,03
average	11,08	4,19	69,59	806,28	20,28	19,12	7506,06

The development of an efficient breeding program to increase mineral concentration in maize depends on the presence of genetic variability in this species (Menkir, 2008). The Mg, Fe, Zn, and P content of 74 maize inbred lines are presented in Table 1. The concentration of Mg varied from 587,81mg/g to 980,62mg/g, Fe from 2,31 mg/g to 28,97mg/g, Zn from 10,84 mg/g to 34,59mg/g, and P from 5315,21ug/g to 8706,27ug/g. The highest average concentration of Mg, Fe and P had inbred lines from European germplasm while inbred lines from BSSS germplasm had the highest Zn concentration. Lines from BSSS had the lowest average Mg and Fe content as well as lines from Lancaster germplasm had the lowest Zn and P content. Menkir (2008) found similar variation in Fe, Zn, and P concentration in 278 maize genotypes and showed that there were highly significant effect of maize genotypes in mineral content. Queiroz *et al.* (2011) found significant genetic variability in the contents of Fe, Zn and P in 22 maize inbred lines.

### Conclusion

Adequate macro and microelements concentration in seed is very important for crop productivity and nutritive value of product. Considerable variability for macro and microelements in grain of 74 maize inbred lines was determined. Inbred lines from European germplasm have the highest protein, oil, Mg, Fe and P content, as well as lines from BSSS germplasm have the highest starch and Zn content and lowest Mg and Fe content. Lines from Lancaster germplasm have the lowest Zn and P content.

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## **SPIKE TRAITS VARIABILITY IN WHEAT GROWN ON SOLONETZ AND HUMOGLEY**

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### **Abstract**

The utilization of less productive soil could be one of the ways to enhance food production. That kind of soil under amelioration and suitable cultivar could give economically sound results. Wheat, being a durable cultivar could be used for agricultural production in less productive soil conditions. Moreover, single plant trait variability became increasingly important in abiotic stress conditions. A phenotypic variability for spike parameters was studied in trial established on two soil types – humogley and solonetz. Humogley exhibits less favorable water-physical properties, while solonetz represents sodium reach, alkaline soils with a subsurface clay horizon. Familiarization with wheat plant behavior in those agro-ecological conditions could be of help for special purposes wheat breeding or selection of suitable wheat varieties among existing genetic variability.

**Key words:** wheat, solonetz, humogley, spike traits

### **Introduction**

Growing of bread wheat in order to provide food for about 70% of the world population, is one of the main tasks of intensive agricultural production. The fulfillment of this task is made difficult by global climate change, excessive use of chemical pesticides, inappropriate use of fertilizers and irrigation, leading to the degradation of arable land. The consequence is a phenomenon abiotic stress conditions affecting plants outdoor research and production. The study of bread wheat growing conditions in abiotic stresses that are less productive soils, contributes to the existing genetic variability selection of the genotypes suitable for growing in that conditions, increasing the usability of these soils, as well (Petrovi *et al.*, 2010).

About 81% is of the total area in Vojvodina belongs to agricultural land. The share of arable land is about 75% (Mili *et al.* 2011). Most of the arable land in Vojvodina are chernozem and humogley (about 80%), the rest are less productive soils. Solonetz belongs to halomorphic soil type that is characterized by unfavorable physical and chemical properties, high clay content in the B horizon, high content of sodium, and consequently alkaline reaction. Therefore, solonetz is commonly utilized as natural pasture non convenient to intensive farming. Somewhere in the neighborhood of 80,000 hectares in Vojvodina is classified as this type of soil, mostly in the Banat region (Beli *et al.*, 2012). Soil salinity has negative effect on most crops, but its utilization is possible by the application of ameliorative measures that could enhance the utility value of the land. Solonetz is subjected to chemical and physical meliorative measures. The chemical melioration measure frequently used is application of phosphogypsum. That way, calcium ions replace the harmful sodium ions that could be flushed by physical measure of soil drainage. Small grains including bread wheat are moderately tolerant to increased salt content in soil (Witcombe *et al.*, 2008).

Hydromorphic black soil (humogley) is characterized by an occasional or permanent water saturation. Humogley generally have heavy mechanical composition and unfavorable water-air properties, but is not considered as a less productive soil, because of its chemical

properties that are favorable, the humus 3-6%, high adsorption capacity and high degree of saturation, mainly calcium ions. However, in areas under hydro-technical reclamation (flood prevention and lowering of groundwater level) is suitable for intensive agricultural production (Škori , 1986).

The aim of this study is to investigate genetic variation for spike properties as important yield components in normal and abiotically stressfull experimental environments in order to select varieties of positive reaction to solonetz amelioration, within the existing genetic variability.

### Materials and methods

The study included ten wheat cultivars (*Triticum aestivum ssp. vulgare*,  $2n=6x=42$ ) created in Institute of Field and Vegetable crops in Novi Sad: Renesansa, Mina, Sofija, Sara, Zlatka, Tiha, Anastasija, Pesma, Pobeda and Partizanka. The experiment was set in Pannonian Plain-Banat region, at the locality Kumane (45,54° N, 20,23° E, altitude 73 m) on two soil types: halomorphic soil (solonetz ameliorated by 25 t/ha phosphogypsum) and hydromorphic black soil (humogley). In 2003/2004 the wheat cultivars were planted in a randomized complete block design in three replicates in 2m long rows, 20cm space between rows, 10cm space between plants in the row and 50kg NPK 15:15:15 fertilizer applied. A phenotypic variability for three spike parameters was analyzed: spike length (cm), grain number per spike and grain weight per spike (g). These traits were analyzed at full physiological maturity, and the base sample consisted of 10 plants per replication.

Variability of cultivars focusing on the genotype by environment interaction (G x E) was tested using AMMI (Additive Main Effects and Multiplicative Interaction) analysis by Zobel *et al.* (1988). Data processing was performed in GenStat 9th Edition (2006) VSN International Ltd (www.vsn-intl.com).

### Results and discussion

Abiotically stressful environment brings individual plant properties into the foreground, diminishing intravarietal variation and enhancing genotype by environment interaction (Den i *et al.*, 2000, Dimitrijevi *et al.* 2012). Consequently, three spike traits are discussed in this study. All three of them, spike length, grain number and grain weight per spike could be of the importance for grain yield formation in utilization of less productive soil. The trial has been established on halomorphic solonetz and humogley soil, parallel. The geographical distance of these two soil types was less than a mile, and the same agricultural practice was applied, so the main difference was expected to be due to soil type diversity. The amount of 25t ha<sup>-1</sup> of phosphogypsum has been applied as a measure for solonetz chemical amelioration, that gave a good effect on wheat grain yield components in previous investigations (Petrovi *et al.*, 2009). According to the results, ameliorated solonetz appeared to be of similar productive level as neighboring humogley. There were no significant differences in overall environmental means of spike length regarding soil type ( $\bar{x}$ =8.0cm, and  $\bar{x}$ =8.4cm, respectively). Some differences at a trend level were denoted comparing environmental means for grain number per spike, where amelirated solonetz part of the plot was slightly more fruitful ( $\bar{x}$ = 33 grains/spike on solonetz vs.  $\bar{x}$ = 29 grains/spike on humogley). Statistically significant difference was observed for the most sensitive mass trait – grain weight per spike. The spike yield was higher on ameliorated solonetz ( $\bar{x}$ =1.4g) than on humogley ( $\bar{x}$ =0.9g), tab. 1.

Table 1. Spike length (cm), grain number per spike and grain weight per spike (g) of 10 wheat cultivars grown in two environments- solonetz soil ameliorated by 25t ha<sup>-1</sup> of

phosphorgypsum (S) and humogley (H). Mean values ( $\bar{X}$ ), treatment variance ( $\sigma^2$ ) and interaction PCA are given

Trait	Spike length [cm]				Grain number/spike				Grain weight/spike [g]			
	S	H	$\bar{X}$	IPCA <sub>G1</sub>	S	H	$\bar{X}$	IPCA <sub>G1</sub>	S	H	$\bar{X}$	IPCA <sub>G1</sub>
Rebensansa	8,1	8,1	8,1	-0,410	33	29	31	-0,017	1,3	1,0	1,2	0,199
Mina	7,4	7,9	7,7	0,011	31	24	27	-0,850	1,3	0,6	1,0	-0,273
Sofija	8,7	9,2	9,0	0,089	28	25	27	0,317	1,3	0,7	1,0	-0,172
Sara	7,6	7,9	7,7	-0,121	35	31	33	-0,100	1,4	1,0	1,2	0,030
Zlatka	6,9	8,0	7,5	0,509	29	33	31	1,901	1,3	1,2	1,3	0,367
Tiha	7,7	8,6	8,1	0,323	34	34	34	0,734	1,3	0,9	1,1	0,064
Anastasija	7,7	7,6	7,7	-0,383	37	27	32	-1,434	1,6	0,9	1,3	-0,306
Pesma	9,8	9,9	9,8	-0,252	36	29	32	-0,934	1,5	0,8	1,2	-0,205
Pobeda	8,0	8,7	8,4	0,194	33	30	31	0,150	1,4	1,0	1,2	0,030
Partizanka	8,0	8,5	8,3	0,037	31	28	29	0,233	1,2	1,0	1,1	0,266
$\bar{X}$	8,0	8,4	8,2	-	33	29	31	-	1,4	0,9	1,1	-
$\sigma^2$	0,75	0,74	-	-	19,4	23,6	-	-	0,07	0,07	-	-
IPCA <sub>E1</sub>	-0,64	0,64	-	-	2,00	-2,00	-	-	-0,50	0,50	-	-
	LSD <sub>0,05</sub> =4,947		LSD <sub>0,01</sub> =6,619		LSD <sub>0,05</sub> =4,947		LSD <sub>0,01</sub> =6,619		LSD <sub>0,05</sub> =0,291		LSD <sub>0,01</sub> =0,389	

AMMI analysis of variance (ANOVA) identified and quantified the sources of phenotypic variation in trial (Zobel et al., 1988, Dimitrijevi & Petrovi, 2005). All three main sources of variation aggregated in treatments variation, appeared to be of significant mean square (tab. 2).

Table 2. AMMI ANOVA for the spike length (cm), grain number per spike and grain weight per spike (g) of 10 wheat cultivars grown in two environments- solonetz soil ameliorated by 25t/ha of phosphorgypsum

Source of variation	Trait	Spike length		Grain number/spike		Grain weight/spike		F-tab.		
		df	MS	F-calc.	MS	F-calc.	MS	F-calc.	0,05	0,01
		Trial	59	0,784	-	24,39	-	0,121	-	-
Treatments	19	1,718	**6,00	36,71	**2,45	0,234	**4,53	1,84	2,37	
Genotypes	9	3,068	**10,71	34,60	*2,31	0,056	1,08	2,12	2,88	
Environments	1	3,083	3,72	194,40	3,85	3,220	**15,58	4,08	7,31	
Block	4	0,829	*2,90	50,53	*3,37	0,207	**4,00	2,61	3,83	
G x E Interaction	9	0,216	0,76	21,29	1,42	0,080	1,55	2,12	2,88	
IPCA 1	9	0,216	0,76	21,29	1,42	0,080	1,55	2,12	2,88	
Trial Error	36	0,286	-	14,98	-	0,052	-	-	-	

Closer analysis reveals that genotypes were significant cause of total variation for spike length and grain number per spike, but quite uniform, in a whole, spike yield. Since, two distinct environments were studied, a non-significant environmental component had been expected. However, the only significant mean square was calculated for the grain weight per spike. Mass parameters with its distinct quantitative nature are the most sensitive phenotypic markers. There was no significant cross over GE interaction, meaning that varieties reacted without rang changes, on average, over environments. The same goes for the one principal

axes that caught all the agronomically explainable. The existence of only one principal component axes in GE variance reveals that the predominant difference in trial was soil type and that wheat varieties based on the same ideotype reacted, on average, in a similar manner to environmental variation. The statistical data obtained using AMMI model ANOVA analysis, basically revealed that chemical amelioration by phosphogypsum combined by proper wheat variety selection out of existing and available gene pool could compensate, judging by yield components in study, unfavorable physico-chemical properties of solonetz soil. However, ANOVA trots out the variation calculated trough phenotypic average behavior of genotypes. Being, basically additive model ANOVA requires additional partition of multivariate GE variance by Principal Component Analysis. The lack of statistically significant and agronomically explainable variance on IPCA1 is just a consequence of similar average behavior of wheat varieties in study for examined spike properties on ameliorated solonetz and humoglay (tab. 2).

Additional insight of individual varietal behavior followed by AMMI model biplot reveals that some wheat varieties were better adapted to humoglay growth conditions, while the others performed better in the environment of ameliorated solonetz. Moreover, a part of the varieties exhibited more stable reaction in both environments, than the others.

The most stable reaction for the spike length was observed for varieties Mina and Partizanka, as well as, Sara and Sofija. On the contrary, varieties Zlatka, Renesansa, and Anastasija appeared to be least stable in given environmental conditions. The best overall performance for the trait showed variety Pesma, being moderately stable at the highest mean value of the spike length. Variety Zlatka was better adapted to humoglay experimental environment, while varieties Anastasija and Renesansa gave better general performance in the environment of ameliorated solonetz (fig. 1).

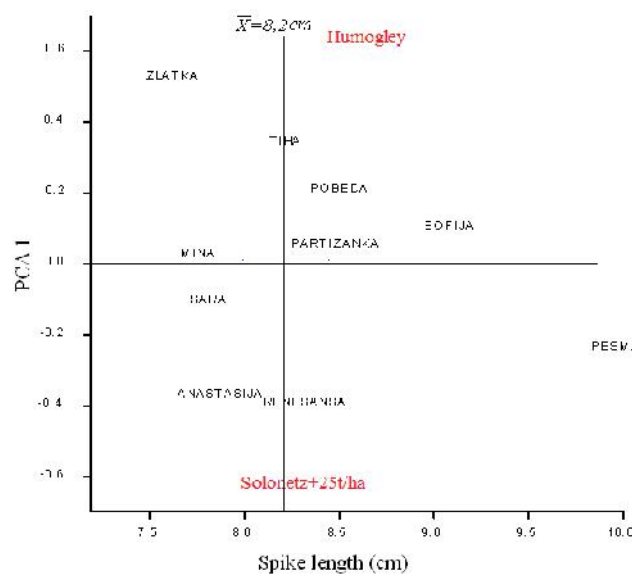


Figure 1. Spike length (cm) of 10 wheat cultivars in 2 environments grouping after mean and PCA1 values

Varieties Renesansa and Sara, followed by Pobeda, Partizanka and Sofija were of the most stable reaction over the environments, for the grain number per spike. Once more, variety Zlatka performed the best for the trait in humogley experimental surrounding, while variety Anastasia preferred ameliorated solonetz as a soil substrat (fig. 2).

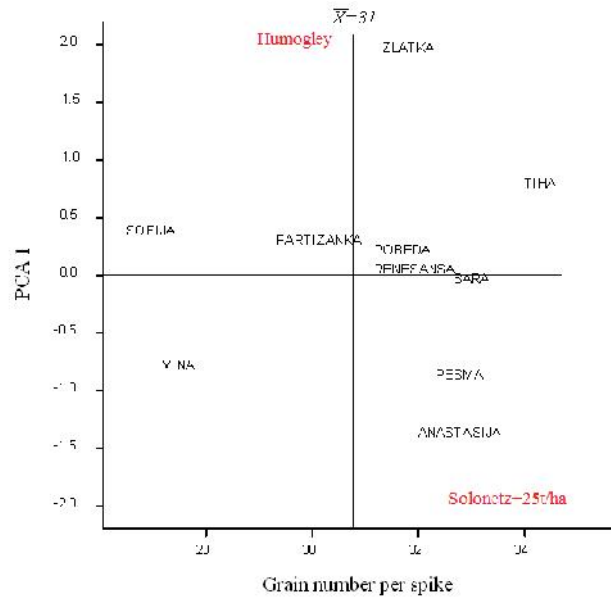


Figure 2. Grain number per spike of 10 wheat cultivars in 2 environments grouping after mean and PCA1 values

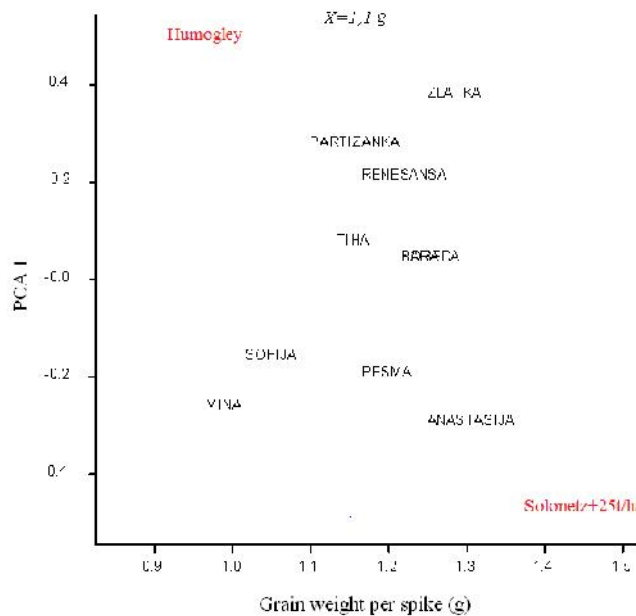


Figure 3. Grain weight per spike of 10 wheat cultivars in 2 environments grouping after mean and PCA1 values

A spike yield given through the grain weight per spike was of particular interest because it aggregates the performance of all plant traits. The most stable reaction for the trait was exhibited by varieties Sara, Pobeda, and Tiha, as well. Anastasija appeared to be of the best performance in ameliorated solonetz environmental conditions, while variety Zlatka, gave the best results among the studied group in humoglay experimental environment (fig. 3).

### Conclusion

Results suggested that there is a possibility of the production performance improvement of wheat varieties grown in extremely abiotically stressful conditions of solonetz, to the level of normally productive humoglay, by chemical amelioration of solonetz and selection of wheat varieties within the existing genetic variability. In a trial that predominantly differed in soil type, it was possible to select genotype of desirable variation for the spike length, grain number and weight per spike. Variety Zlatka performed better in humoglay experimental environment, while variety Anastasia preferred ameliorated solonetz conditions.

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## OLIVE MULTIPLICATION IN DIFFERENT PHASES OF MERISTEMATIC DEVELOPMENT

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### Abstract

The object of this research is propagation through green macro explants derived from the apical segment of the sprig in the variety “Kaninjot, Mixan, Bllanic and Himara. The parts were treated with Indole-3 Butyric Acid,  $1\text{g l}^{-1}$ ,  $3\text{g l}^{-1}$ ,  $5\text{g l}^{-1}$  and Control in four phases of the meristematic development; February ( $\frac{1}{2}$  asleep), May (active), September (active) and December (asleep).

Thermal regimes  $24^{\circ}\text{C}/18^{\circ}\text{C}$  ( $\pm 1^{\circ}\text{C}$ ), the mist technique was applied according to Photo synthetically Active Radiation  $5\text{ sec}/11\text{-}13\text{ k.kal/cm}^2$ .

After 70 days, the percentage of rooting of studied cultivars varies from 23 to 90%. The two IBA dosages have not affected rooting in the same way within each period. IBA at  $3\text{ g l}^{-1}$  and  $5\text{ g l}^{-1}$  gave high rooting percentages for Kaninjot and Mixan cultivars. At high cambial activity, the IBA concentration of  $3\text{g l}^{-1}$  resulted more effective; whereas under conditions of low cambial activity, high IBA concentrations yielded better results. At active vegetation stage the IBA in high concentrations shows inhibitory and toxic effect. The concentration of  $1\text{g l}^{-1}$ , had weak reactions and was inefficient. The control gave low rooting percentage and with significantly highlighted changes compared to IBA treatments. Cultivars have their highest endogenous rhizogenic capacity in May and September, period which corresponds to the active cambium activity. Defoliation has varied between 8.3 and 14.1%. The presence of the leaves has been a stimulating factor for rooting ( $r^2=0.93$ ). The number of roots has increased in parallel with the increase of concentration, ( $r^2=0.72$ ).

**Keywords:** Olive, cultivars., mist-propagation, explants, hidroalcoholic, culture.

### Introduction

The "mist propagation" technique, has a high propagation coefficient, and produces a powerful and resistant seedling to diseases, but its efficiency is influenced by different factors such as endogenous hormonal stimulants (Hartmann, 1952) . exogenous hormonal stimulants (Leva et al.,1999). hormonal acid concentrations (Bartolini et al. 1989) the nature of the green cutting and its place in the spring the time applied for propagation, etc. (Caballero, 1983.) A lot of researches for the propagation of the olive varieties have proved different rooting capacities from one variety to the other, Fabri (1980). Meanwhile a lot of hormonal acids have been experimented with several hydroalcoholic concentrations and the obtained results have varied according to the genotype used. Due to these reasons, the research is mainly oriented on the study of the effect of the indol-3 butyric acid in extreme concentrations and in the typical vegetation phases: February, May, September and December for 4 main olive cultivars in Albania. in the typical vegetation phases: February, May, September and December



## Materials and methods

Vegetative material: Green cuttings of the four olive cultivars (*Kaninjot*, *Bllanic*, *Himara* and *Mixan*), are tested for their rooting capacity with the "mist propagation" method, in correlation with three concentrations of indol-3 butyric acid (AIB) and (Control), during four stages of their meristematic activity.

Rooting period has been every month on the fifth day. (February, May, September, December). The green cuttings are 8-10 cm long, having two pairs of leaves at the apical, with all the leaves at the base being cut off. Each treatment has 100 cuttings (4 times x 25 cuttings). The 5 sec treatment with hydro alcoholic solution of indol butyric acid (ecyberon) in two concentrations of  $1 \text{ g l}^{-1}$ ,  $3 \text{ g l}^{-1}$  and  $5 \text{ g l}^{-1}$ .

A Control treatment has been considered and applied as comparative. In every hydro alcoholic concentration, the percentage of alcohol is 24% and H<sub>2</sub>O 76%.

Temperature and Humidity regimes: The cuttings have been planted at nebulization bank. within the biological green house, with perlite substrate. Temperature has been kept at 20°C and at 24°C ( $\pm 1^\circ\text{C}$ ) in the substrates. Nebulization has been achieved for 5 sec at every 15 Wh/m<sup>2</sup>, depending on the sun radiation. Illumination at the bank is 6000 lux. Solar integrator module has been set to be automatic (Gonda et al 2006).

Statistical analysis: It has been carried out by means of software modeled for 100 green cuttings for Rooting percentage (rooted cuttings in %), defoliation percentage estimated for each cultivar in (%). The Variance Analysis for Tukey-Kramer test, (P= 0.05) has recognized and certified the differences of each treatment. (Jmp.Sas/Stat.2008)

## Results and Discussion

The process of rhizogenesis: Rhizogenous processes during 7 weeks in a nebulisation bank, display a lot of modifications on the top and bottom of the green cutting. These were the result of the separation from the parent tree, the application of auxins, base warming and vaporization, to which the cuttings were in the regime. On the top, a considerable part of the cuttings has lost one or several leaves. In the segments of the cuttings base, during the two first weeks the wound in the base was healed, whereas two weeks later there was intensive propagation of cells and the formation of an obvious cellular mass, colored white -into - cream called a "Callus

The differentiation of meristem radicles starts immediately after the creation of the callus, depending on the cultivar, the concentration, and the period of time applied for rooting. The cuttings which dropped the leaves, have been associated with callus degeneration and the canker of the substrate tissue. An important cause of the fallen leaves were the infections of *Cyloconium oleaginum* and the physiological state. The presence of leaves on top of the green cutting was a really important factor for the success of rooting, especially for the creation of the roots emission (Hartmann, 2002; Ciampi, C. et al.1958; Bouillenne, et al 1933).

The feedback of the olive varieties in function of the IBA dose and period of time. Some cultivars have registered extreme values of the rooting percentages in different concentrations of the hormone. It was proved that not all three treatments affected in the same way the rooting result of the olive cuttings at any tested term. Both concentrations of the Indole -3- Butyric Acid (IBA), have influenced differently. Rooting percentage is considerably obvious, not only for the dose  $3 \text{ g l}^{-1}$ , but also for  $5 \text{ g l}^{-1}$ , whereas for other varieties there has been either one dose or the other with regard to only one typical rooting term. The Kaninjot cv has had an extremely high rooting percentage in both concentrations:

with IBA  $5g\ l^{-1}$  (78.6% in February and 72% in September). But also with IBA  $3g\ l^{-1}$  it rooted (71-0% in February, 89.0% in May, and 57.0% in September). A high rooting percentage has also resulted for cv. Mixan. with IBA  $5g\ l^{-1}$  55.3% in February in may and 56% in September while with IBA  $3g\ l^{-1}$  57% in may). Whereas the cultivars; *Bllanic and Himara*, have displayed low rooting percentage (Table 1).

The above mentioned cultivars although displaying sufficient callus size, after 30-35 days, their callus increases in volume, and becomes like a spongy mass, that it can't reach rooting. Non-differentiation of rooting is a genetic characteristic and occurs because of lack of enzyme activators that synthesize the auxinic complexes, assimilated by the phloem in these cultivars (Caballero, 1983).

Table 1. The effect of both IBA concentrations and the period on rooting percentage, defoliation percentage and number of roots

Teeatment	February			May			September			December		
	Rooting %	N° leaves	N° Roots	Rooting %	N° leaves	N° Roots	Rooting %	N° Leaves	N° Roots	Rootin g %	N° Leaves	N° Roots
Kaninjot/ Control	35.0 klmn	2.1 fghi	3.2 ef ghij	31.0 mno	1.86 ijk	4.3 bc defgh	29.0 no	1.86 ijk	3.3 ef ghij	19.0 p	2.0 hij	2.33 g hijkl
Bllanic/ Control	0.0 w	0.33 nopq	0.0 l	4.0 stuvw	0.96 lmnop	2.33 g hijkl	0.0 w	0.13 q	0.0 l	0.0 w	0.46 mnopqr	0.0 l
Himara/ Control	0.0 w	1.16 klm	0.0 l	2.0 uvw	1.06 lmn	1.33 ijkl	0.0 w	0.26 opq	0.0 l	0.0 w	0.23 pq	0.0 l
Mixan /Control	27.0 o	2.4 bcfghi	4.0 bc defg	29.0 no	2.23 efghi	4.6 bc defg	11.0 qrs	1.2 klm	2.6 fg hijk	11.0 qrs	1.03 lmno	2.66 f ghijk
Kaninjot/ IBA $1g\ l^{-1}$	47.0 ghi	2.0 hij	5.33 abcde	49.0 fgh	2.53 bcfghi	4.66 b cdefg	49.6 fgh	2.53 bcfghi	4.3 b cdefgh	38.3 jkl	2.0 hij	3.0 ef ghij
Bllanic/ IBA $1g\ l^{-1}$	7.3 rstuv	0.66 mnopq	2.33 g hijkl	9.6 rst	1.2 klm	2.66 f ghijk	1.6 vw	0.3 nopq	1.0 jkl	0.0 w	0.26 opq	0.0 l
Himara/ IBA $1g\ l^{-1}$	4.3 stuvw	0.46 mnopqr	3.0 ef ghij	11.0 qrs	1.06 lmn	2.66 f ghijk	0.0 w	0.2 pq	0.33 kl	0.0 w	0.36 Nopqr	0.0 l
Mixan/ IBA $1g\ l^{-1}$	39.0 ghij	2.1 ghij	6.3 abc	51.0 efg	2.4 cdfghi	4.3 bc defgh	41.0 hij	2.46 bcfghi	3.6 de fghi	32.0 lmno	2.2 ghijl	3.3 ef ghij
Kaninjot/ IBA $3g\ l^{-1}$	71.0 bc	3.03 abcd	6.0 abcd	89.0 a	3.43 a	6.66 ab	57.0 de	2.66 bcdefg	4.0 cd efgh	41.0 ijk	1.33 jkl	3.6 de fghi
Bllanic /IBA $3g\ l^{-1}$	6.0 rstuvw	0.33 nopq	3.3 ef ghij	13.0 pqr	0.76 mnopq	3.3 ef ghij	2.0 uvw	0.56 mnopq	2.33 g hijkl	0.0 w	0.46 mnopqr	0.0 l
Himara/ IBA $3g\ l^{-1}$	4.0 stuvw	0.3 nopq	2.33 g hijkl	13.0 pqr	0.66 mnopq	3.0 ef ghij	5.0 stuvw	0.73 mnopq	1.33 ijkl	0.0 w	0.13 q	0.0 l
Mixan/ IBA $3g\ l^{-1}$	50.0 efgh	2.3 defghi	2.66 f ghijk	57.0 de	2.9 abcdef	7.3 a	43.3 efgh	2.83 abcdefg	5.3 abcde	38.0 jklm	2.73 abcdefg	3.6 de fghi
Kaninjot/ IBA $5g\ l^{-1}$	78.6 b	3.2 ab	6.33 abc	68.0 cb	3.03 abcd	6.33 abc	72.0 bc	3.16 abc	4.3 bc defgh	46.0gh i	2.8 abcdefg	3.6de fghi
Bllanic/ IBA $5g\ l^{-1}$	7.0 rstuvw	0.93 lmnop	2.33 g hijkl	7.0 rstuvw	0.86 mnopq	2.33 g hijkl	3.0 tuv	0.73 mnopq	2.33 g hijkl	0.0 w	0.2 pq	0.0 l
Himara/ IBA $5g\ l^{-1}$	9.0 rstu	0.6 mnopq	3.3 ef ghij	17.0 pq	0.96 lmnop	4.3 bc defgh	1.0 vw	0.73 mnopq	2.0 hijkl	0.0 w	0.26 opq	0.0 l
Mixan/ IBA $5g\ l^{-1}$	55.3 ef	3.0 abcde	5.33 abcde	52.0 efg	2.56 bcfghi	6.0 abcd	56.0 def	2.9 abcdef	5.0 ab cdef	35.0 klmn	2.03 hij	3.3 ef ghij

Levels not connected by same letter are significantly different. Rooting %. lsd.1.12hsd. N° of Roots -lsd. 1.03hsd and N° of Leaves -lsd.1.31 hsd. ( $P=0.05$ ).

In Tab 1. The effect of both IBA concentrations on rooting percentage; and the relation to the six cultivars seems to have stimulated considerably different concentrations. Through the variance of the values (*lsd 1.12 HSD, Alpha=0.05*), it resulted as follows: In February; *Kaninjot IBA 5 g l<sup>-1</sup>*, displays the highest rooting percentage of 78.6%, with a dominant position compared to other treatments. Whereas the treatment *Kaninjot IBA 3 g l<sup>-1</sup>* 71% as well as the treatment. In May the treatment, *Kaninjot IBA 3 g l<sup>-1</sup>* display a value of 89% displayed a higher rooting value compared to treatments with IBA 5 g l<sup>-1</sup> and the Control. Two varieties: *Bllanic* dhe *Himara* have manifested lower rooting ability compared with *Kaninjot* and *Mixan* variety. In September: there are the treatments with IBA 3 g l<sup>-1</sup>, which have a higher rooting percentage as well as a more favorable and dominant position than IBA 5 g l<sup>-1</sup>. In December: All treatments displayed lower rooting capacities compared to the other terms. The use of the AIB 5 g l<sup>-1</sup> in any cultivar displayed dominance compared to the 3 g l<sup>-1</sup> and 1 g l<sup>-1</sup> concentration.

The interconnections between the time of propagation and the hormone in different concentrations, displayed strong correlative relations and have a dominant position for the period of February and December with concentration 5g l<sup>-1</sup> ( $r=0.712$ ) and May/September with concentration 3 g l<sup>-1</sup> ( $r=0.908$ ), i.e.: when there is intensive vegetative flux there is higher rooting percentage.

In any case the difference between the percentages achieved with the dose 3 g l<sup>-1</sup> and 5 g l<sup>-1</sup> is not significant except for *Kaninjot cv* and *Mixan cv*. But if we focus the analysis towards the differences between the varieties, it can be noticed that the *Kaninjot cv* has given the highest rooting percentage for the three doses of the IBA applied (89%), followed by *Mixan cv*. (55.3). The treatment 1 g l<sup>-1</sup> have manifested average results with significant changes, with the other treatments. Only *Bllanic cv* and *Himara cv*, displayed really low percentages (less than 20%), in both concentrations and in the four application periods for rooting. The really high percentage of the callogenesis in *Himara cv* and *Blanic cv*, has been considerably different and opposite to the low rooting percentage achieved in these two varieties, (*Ismaili H. et all. 2011*).

The cultivars have had a better promontory rooting activity during the period of spring – summer compared to winter, and in correlation with the concentrations it seems that the endogenous equilibriums are different for each cultivar. More efficient are the cultivars 3 g l<sup>-1</sup> and 1 g l<sup>-1</sup> when there is vegetative growth and active cambium activity; on the contrary when the cambial activity is low high concentrations of the IBA have resulted in better results. There have been several cases (*Vegetative Flux*), when the IBA in high concentrations has resulted limiting or toxic. *Fabbri, A. 1980*.

The effect of leaves resistance. The leaves have had an important role in the life persistence of the cuttings and rooting emission, because they constitute the only source of nutrition during their stay in the nebulisation bank. Generally it has resulted that all the cuttings which have emerged roots have preserved the leaves, whereas those which have dropped the leaves do not have differentiated roots, *Bartolini et all. 1989*. After root emission there was a low percentage of cuttings which dropped the leaves and this phenomenon was a pathogens cause, or because of excessive humidity, *Fernandes S. et all. 2004*.

Table 1 and figura-3a, display the importance of leaves resistance for the process of rhizogenesis, which give the necessary energy for the formation of roots. Most of the cuttings

preserve the totality of their leaves depending on the applied concentration of the cultivars. For example Kaninjot cv in both IBA concentrations, has had cuttings with 4 leaves, 87%, and 75% whereas the cultivars with a low rooting percentage have been associated with high defoliation percentage. The Kaninjot cv displays the lowest percentage of cuttings mortality, whereas the Bllanic cv and Himara cv, the highest percentage. In the concentration  $5 \text{ g l}^{-1}$  about 50% of the green cuttings have preserved the number of leaves 100%. The most exaggerated foliage has corresponded to the control.

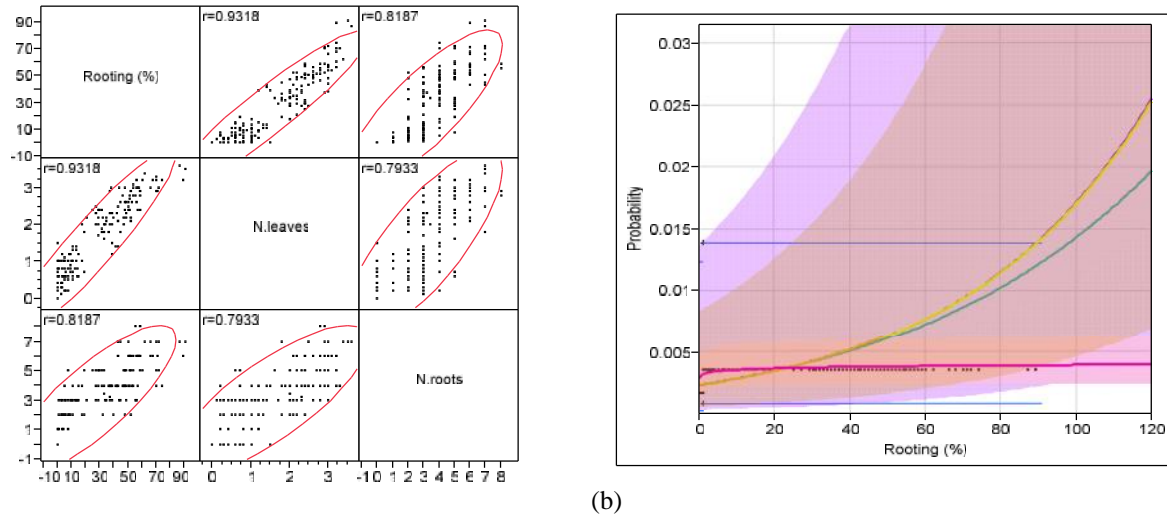


Figure-2, (a) Multivariate Pairwise Correlations estimated by REML method for rooting (%), N.leaves, n.roots and treatments. (b) Regression orthogonal for distribution of rooting (%) for different propability, about variety, period and the concentration of IBA, analyzed the averages of four genotypes of olive.

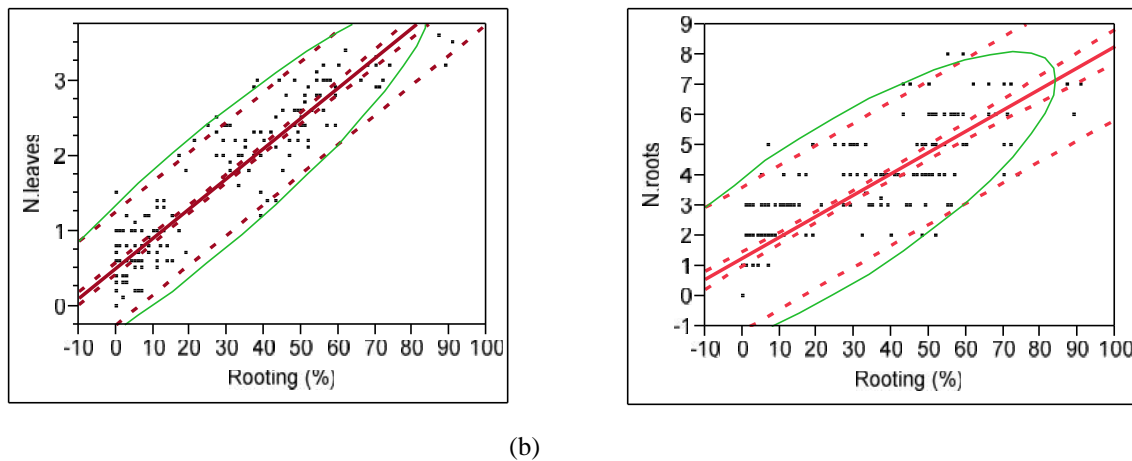


Figure-3 (a) Regression orthogonal for propability of interdependence and distribution of points in realized regression of number.leaves by rooting (%) and (b) number roots by rooting (%), (the averages of four genotypes of olive). In these plots, points that are not on the border of credibility, ( $P=0.005$ ) are not reliable.

The underlined results in *table-1*, show that the persistence of rooting in 9 weeks is indispensable to achieve the ability of the green cuttings to root. Whereas the green cuttings of the Himara cv and Bllanic, despite having preserved the leaves, have undergone modifications of the callus quality, degeneration in a spongy and hyperplastic form which have been followed by low rooting percentage, *Fabbri, A. et all. 2004*.

The correlations have displayed the connection between rooting percentage and defoliation percentage, ( $r^2=0.93$ ), meanwhile between the rooting percentage and numbers of

roots has strong links ( $r^2=0.81$ , but the relationship between of numbers of leaves and roots is weak ( $r^2=0.79$ ). In any case the application of excessive vapor has caused a high percentage of defoliation expressed in (%). I.e.; a high rooting percentage has had a low defoliation percentage and the opposite; in any case the high percentage of the green cuttings defoliation is accompanied with low rooting. Based on three-years averages, the distribution of values were rooting percentage of probability between 0.005 and 0.001, making no statistically significant average below 32%.  $\lambda=0.1$  table-1 and Figure-2ab.

Ne figuren-3 (a.b) Probability of interdependence Bivariate Fit of N. leaves By Rooting (%), turns out that the rooting percentage increase goes in parallel with the number of leaves on green cuttings ( $r^2=0.93$ ) and number of roots by rooting (%), again in the walking connection and parallel growth ( $r^2=0.79$ ).

The values presented graphically in fig.7, express the rhizogenic average percentage of the cultivars. It is noticed that they change considerably under the influence of the season when they take the material for propagation. In the diagram it is obvious that the natural percentage values begin in February, a period when vegetation begins, and are weakened later by fifty up to the end of the vegetative cycle in December.

The results showed that vegetative growth of the olive has influenced on the rooting percentage. The best rooting results are presented with the time as soon as vegetative growth becomes intensive, and coincide with the moments when vegetative growth is inhibited, the values of rhizogenesis are limited, thus undergoing a considerable decrease which coincides to the phase that trees mature their fruit. This phenophase corresponds when the inhibitors, especially the phenols are with the highest concentrations (Leva et. all. 1999).

High rooting percentage is noticed during the period of intensive vegetative growth. The rhythms of vegetative growth begin, become intensive and are gradually reduced until they reach point zero during winter hibernation. Although a slight increase is noticed in March 0.7 mm/day rooting results are high, because the level of the natural promoters for rooting begins to become active. The three concentrations of IBA and their relation to the phonological season show the efficiency of concentration  $3 \text{ g l}^{-1}$ , when the endogenous capacity is maximal (May-September) and concentration  $5 \text{ g l}^{-1}$  is more efficient when the promoters fail, or are in minimal quantity.

### Conclusion

The process of rhizogenesis of the green cuttings of the olive is a complicated phenomenon which aims at the formation of a new plant capable of growing independently.

The experiments carried out so far, prove the existence of a correlation among the cultivars, the concentrations and period of time applied for rooting. The olive “with nebulisation” displayed an obvious sensibility considering ripeness of the cuttings in all cultivars.

In February and May there was a higher rooting percentage more than 50%, thus making it convincing that the nebulisation method is efficient. The cultivar Bllanic, had an average rooting percentage in May, whereas in the other terms it displayed a low rooting percentage. The cultivar Himara displayed low rooting percentage in all the four experimented terms.

The dose of IBA;  $3 \text{ g l}^{-1}$  and  $5 \text{ g l}^{-1}$  stimulated high rooting percentages, but also in correlation with the period of treatment.

The doses of IBA  $3 \text{ g l}^{-1}$  and  $5 \text{ g l}^{-1}$ , were efficient in correlation with the period when they were used, thus giving a maximal increase of the rooting percentage and leaves resistance up to the end of the rooting process.

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## EFFECT OF AGRO-ECOLOGICAL CONDITIONS ON GRAIN YIELD IN SOME GENOTYPES OF BUCKWHEAT

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### Abstract

Buckwheat (*Fagopyrum esculentum*) is an important field crop in our mountainous areas. It has long been neglected, but more recently, the area under buckwheat has increased. There is no official data on the areas where buckwheat is grown, or what are its average yields in Republic of Srpska.

In the two-year period, our research has included four varieties of buckwheat (Gray, Darya, Bednja and Novi Sad) at two sites (Sarajevo and Sokolac). On the basis of the survey, data confirms the hypothesis that the buckwheat plant is suited to more humid regions, because of the higher yield at the sites and years that were richer with precipitation. Year 2012 was with unfavorable sum and distribution of precipitation and high air temperatures which resulted in average yield and other quality characteristics of the tested varieties of buckwheat. Varieties Darya and Bednja in extreme conditions achieved the same average yields. In both years of testing these varieties had significantly higher yields compared with gray and Novi Sad varieties of buckwheat.

**Key words:** buckwheat, variety, temperature, precipitations, yield.

### Introduction

Buckwheat (*Fagopyrum esculentum*) is an important field crop in our mountainous areas. It has long been neglected, but more recently, the area under buckwheat has increased. There is no official data on the areas where buckwheat is grown, or what are its average yields in Republic of Srpska.

Buckwheat is grown for its fruits, which hulled have great nutritional value. Due to its favorable chemical composition, buckwheat is suitable in nutrition of diabetics and children. Nutritionists classify it in the group of plants suitable for the production of biologically valuable food and as such they have declared it a health beneficial food.

Peeled fruit of buckwheat contains 9.07% of total protein, 70.98% NFE, 3.7% cellulosic material, 1.73% oil, 1.72% mineral salts I2, 8% water (*Glamo lija*, 2004).

It is of particular importance for people with diabetes due to the fact that the food of buckwheat grain influences the reduction of the concentration of sugar and fat in the blood. The buckwheat biomass above the ground there is bioflavonoid rutin, which is of great importance in the pharmaceutical industry to obtain drugs that lower blood pressure, stop capillary bleeding, and reduce cholesterol in the blood. Because of these qualities buckwheat was declared a medicinal herb of the year in Germany in 1999 (*Gadžo*, 2009).

Buckwheat belongs to the group of melliferous-honey plants. The flowers are rich in nectar and blooming lasts for a long time, which is excellent bee pasture. Buckwheat has a great importance for soil management; as dense crop it covers the land and suppresses weeds.

The aim of our studies was to determine the effect of agro-ecological conditions on grain yield in some genotypes of buckwheat.

### Material and method

Three factorial experiment included the effect of variety (A), year (B) and location (C) on the yield of buckwheat. The experiment consisted of testing four varieties: Gray, Darya, Bednja and Novi Sad. Sowing buckwheat was performed on 20th April 2011 and 23 April 2012 in Sarajevo, and 08th May 2011 and 10 December 2012 at Sokolac.

Area of elementary plot was 12 m<sup>2</sup> (4 m long and 3 m wide). Trials were conducted in five replicates on private farms in two locations: in the Romanija near Sokolac at an altitude of 872 m and in Sarajevo Field (Lukavica) at an altitude of about 550 meters. Common cultural practices that are used in the cultivation of buckwheat were applied.

Buckwheat was harvested on 25 July 2011 and 20 July 2012, and on 14 August 2011 and 11 August 2012 in the area of Sarajevo and Sokolac respectively. Buckwheat grain yield was determined by measuring each basic plot and converted to 12% humidity.

The results were analyzed using analysis of variance for three factorial experiment (ANOVA) using SPSS 4.5 software. The significance of differences in mean values of treatment was tested by LSD test.

The experiment was conducted on brown valley land in Lukavica and sour brown (dystric kambisol) in Sokolac. Soil reaction at both sites is acidic. In Lukavica, the land is moderately secured with phosphorous and potassium, while at Sokolac, it was poorly secured with phosphorus and well secured with potassium.

**Tab.1** Chemical properties of soil

Location	pH		Humus (%)	N (%)	mg/100 g	
					P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
Lukavica	6,28	5,45	2,76	0,17	16,4	19,02
Sokolac	6,22	5,17	5,1	0,34	1,43	34,3

The experiment in Lukavica (Sarajevo area) was set on loamy soil and sandy clays. Sokolac experiment was set up on land belonging to the class of cambic soil type or distric brown soils which are very widespread in the mountains of central Bosnia (Jovandi ,1977).

**Tab.2** Meteorological data (2011-2012)

Location	Year Month		IV	V	VI	VII	VIII	IX
Lukavica	2011	°C	11.0	14.0	18.9	20.5	21.7	19.1
		mm	32.7	103.6	76.3	134.4	4.8	38.9
	2012	°C	10.4	14.7	23.7	24.6	24.5	17.4
		mm	135	125.2	9.3	34.6	25.9	158.6
Sokolac	2011	°C	7.7	11.0	15.8	17.5	18.2	15.5
		mm	42.7	123.0	62.8	82.2	9.2	36.7
	2012	°C	7.4	10.9	18.7	19.5	18.8	16.2
		mm	93.7	205.1	11.6	5.2	7.6	22.4



Sokolac is located at an altitude of 872 meters. The average annual temperature is 6.8 °C, absolute maximum air temperature is 33.6 °C and the absolute minimum temperature is -30.0 °C. Climate of Romanija region, whose center is in Sokolac, is extremely mountainous, with cold winters and cool summers. Growing season begins around the 8th April and lasts until 22 October, or an average of 197 days. It is a period when air temperatures are above 5 °C and when the growing movement of woody plants is observed. During the year, there are about 800 mm of rainfall, of which about 430 mm during the period April-September (*Mi evi*, 1979).

Sarajevo area is under the influence of middle-European continental climate in the north and the Mediterranean climate of the south. These effects, as well as the diversity of terrain, give this area features a moderate continental climate. The average annual temperature in Sarajevo is 9.5°C. Usually the first frost in Sarajevo occurs on 23rd October and the last on 29th April, with an average of 28 days of ice. The warmest month is July with a mean temperature of 19.1°C, and very close to it is August (mean temperature 18.8°C), which can often be even hotter. The fact that autumn is warmer than spring, as well as the delay in temperature extremes that occur in August, results in a considerable maritime influences. Rainfall in Sarajevo occurs in all seasons and all months and average annual distribution is evenly distributed. Average annual precipitation is 919 mm/m<sup>2</sup>.

### Research results and discussion

The average yield of buckwheat (table 3) for tested years, at both sites and for the four tested varieties was 1.819 t / ha.

**Tab.3.** Grain yield of buckwheat (t/ha)

Variety	Location (C)						Average (A)
	Lukavica			Sokolac			
	2011	2012	Average	2011	2012	Average	
Gray (a <sub>1</sub> )	1.943	1.743	1.843	1.652	1.473	1.562	1.703
Darya (a <sub>2</sub> )	2.206	2.148	2.177	1.803	1.603	1.703	1.940
Bednja (a <sub>3</sub> )	2.272	2.165	2.218	1.824	1.603	1.713	1.965
Novi Sad (a <sub>4</sub> )	1.862	1.702	1.782	1.655	1.459	1.557	1.669
Average	2.070	1.939	2.005	1.733	1.534	1.634	1.819
LSD	A	B	C	AxB	AxC	BxC	AxBxC
0.05	0.065	0.050	0.050	0.120	0.120	0.106	0.239
0.01	0.111	0.085	0.085	0.205	0.205	0.282	0.408

Varieties Darya (1.940 t / ha) and Bednja (1.965 t / ha) achieved significantly higher yields compared with varieties Gray and Novi Sad. The effect of genotype on the grain yield of buckwheat indicates that some varieties have a high genetic potential for yield and that yield potential is stable regardless of agro-ecological conditions (altitude, average temperature, amount and distribution of precipitation). These results are in accordance to the results of *Bogdanovi* (1980) and *Mili et al.*, 2013.

In 2011 the average yield of buckwheat was 1.901 t / ha, and in 2012 1,736 t / ha. High temperatures and uneven distribution of rainfall in 2012 influenced very significantly lower yields of buckwheat compared with 2011. Buckwheat achieves better yields in wetter regions and wetter years (*V. Djordjevic, 1961; Bogdanovic M, 1980; Maletic R. and Jevdjovic R, 2003*). For buckwheat, it is very important that there is sufficient rainfall in the stage of flowering and fruiting. The optimum temperature for growth of buckwheat is 20-25 ° C, while the temperature above 30 ° C is unfavorable for development of buckwheat. In strong drought and high temperature there is almost no grain.

In the area of Sarajevo (Lukavica) the average yield was 2.005 t / ha, while at the area of Romanija (Sokolac) it was 1.634 t / ha. Highly statistically significant difference in the yield on the research areas was influenced by weather conditions and time of sowing. In 2011 in the vegetation period (April-September) there was 390.7 mm of rain in Lukavica, and 356.6 mm in Sokolac, while in 2012 there was 488.6 mm in Lukavica, and 345.6 mm in Sokolac which had a significant impact on the yield of buckwheat in years of research. Although in 2012 there was more rain in Lukavica the yield was lower due to the uneven distribution of rainfall.

Variety Bednja proved to be the most suitable for the cultivation at both sites.

In mountainous area, the attention must be paid to the date of sowing as early sowing brings danger of late spring frosts and late sowing has risks of lack of moisture in the flowering phase of buckwheat and entering in the autumn, rainy weather that can affect the uneven grain ripening. Buckwheat should be reaped when more than 2/3 of grain was ripe.

Varieties Bednja and Grey are so prone to shattering and this point has to be taken into account while growing buckwheat and we should not wait for full maturity, especially in mountainous areas because they are exposed to severe weather conditions that can cause tremendous damage.

### **Conclusion**

Based on results of two years in this experiment it can be concluded that the weather conditions had a significant effect on the yield of buckwheat. Year 2012 was with unfavorable sum and distribution of precipitation and high air temperatures which resulted in average yield and other quality characteristics of the tested varieties of buckwheat.

Varieties Darya and Bednja in extreme conditions achieved approximately the same average yields. In both years of testing these varieties had significantly higher yields compared with gray and Novi Sad varieties of buckwheat.

In the mountainous areas it is important to determine the favorable planting date to avoid late spring and early autumn frosts, lack of moisture in the stage of buckwheat flowering and adverse weather conditions in the fall.

By proper selection of varieties and cultural practices high yields of buckwheat can be achieved.

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## EVALUATION OF FRENCH APRICOT CULTIVARS IN THE REGION OF BELGRADE

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### Abstract

The evaluation of 10 introduced apricot cultivars of French origin was carried out in the region of Belgrade over a period of four years (2009-2012). Control cultivar for comparison was 'Hungarian Best'. Average time of flowering was late March and early April, while average time of maturing ranged from June, 26 ('Sylred') to July, 15 ('Helena du Roussillon'), or from 7 days before to 12 days after the 'Hungarian Best'. Compared with the control cultivar, significantly higher yield was achieved in five cultivars: 'Sylred', 'Bergeron', 'Pinkcot', 'Silvercot', and 'Bergarouge', while significantly higher fruit weight was found in four cultivars: 'Silvercot', 'Sylred', 'Polonais', and 'Bergeron'. Among studied cultivars, the best results were shown by 'Sylred', 'Silvercot' and 'Pinkcot', which can be recommended for growing in this region, predominantly for fresh consumption. 'Bergeron' and 'Bergarouge' can also be recommended as cultivars of combined traits, both for fresh consumption and processing.

**Key words:** *Prunus armeniaca*, flowering, maturing, yield, fruit quality

### Introduction

Assortment of apricots in Serbia is characterized by relatively small number of cultivars and short period of harvest. Most apricot fruits are harvested in the season of 'Hungarian Best', or at a short time (about ten days) afterwards. There is particularly a lack of early-season cultivars, maturing in June, and characterized by high quality of fruit.

A lot of work has been done in the world on the creation of new apricot cultivars with improved characteristics, such as better adaptability to different environmental conditions, higher resistance to disease-causing agents, higher yield, and better fruit quality. In the last 20 years more than 500 new apricot cultivars were created (Milatovi , 2013a). The largest number of new cultivars was created in the United States, followed by France, Italy, Romania, China, Czech Republic and Spain. The introduction of new foreign cultivars and their study in Serbian climatic and soil conditions allow better choice of cultivars, and may improve production of apricots.

In France there is a public apricot breeding programme that takes place in the INRA (Institut National de la Recherche Agronomique) in Avignon. More than 20 new cultivars were created under this programme (Audergon et al., 1995; 2006; 2010). In addition, there are several important private breeding programs, which are mostly connected with the nurseries, such as IPS (International Plant Selection), Cot International, Escande, and Star Fruits (Milatovi , 2013b).

The aim of this study was the evaluation of 10 French apricot cultivars of different maturing time. The best performing cultivars will be recommended for growing in the region of Belgrade, as well as in other regions with similar ecological conditions.

### Material and methods

The study was conducted in the apricot collection orchard at the Experimental Station “Radmilovac” of the Faculty of Agriculture in Belgrade during the period of four years (2009-2012). The orchard was planted in 2007. The rootstock is Myrobalan (*Prunus cerasifera* Ehrh.) seedling, training system is Central Leader, and tree spacing is 4.5 x 3 m. All cultivars are represented by five trees.

The study included 10 apricot cultivars: ‘Sylred’, ‘Pinkcot’, ‘Kyoto’, ‘Luiset’, ‘Silvercot’, ‘Polonais’, ‘Rouge de Rivesaltes’, ‘Bergarouge’, ‘Bergeron’, and ‘Helena du Roussillon’. Cultivar ‘Hungarian Best’ was taken as a control.

Flowering was recorded by recommendations of the International Working Group for pollination: start of flowering - 10% open flowers, fool bloom - 80% open flowers, end of flowering - 90% of the petal fall (Wertheim, 1996). The beginning of harvest was recorded as the date of maturing. Fruit characteristics were measured on a sample of 25 fruits per cultivar. Fruit shape index was calculated by the formula: length x length/width x thickness. Soluble solids content was determined by refractometer and total acids content (expressed as % of malic acid) by titration with 0.1 N NaOH. Sensory properties of the fruit (appearance and taste) were evaluated by a five-member jury, scoring the cultivars using the scale from 1 to 5 points.

The obtained data for yield and fruit weight were statistically analyzed using analysis of variance. The significance of differences between mean values was determined using LSD test at 0.05 level of probability.

### Results and discussion

Phenological traits included time of flowering and time of maturing, and the results are shown in Table 1.

Table 1. Phenological properties of apricot cultivars (average, 2009-2012)

Cultivar	Flowering dates			Duration of flowering (days)	Date of harvest	N <sup>o</sup> of days comparing to control
	Start	Full	End			
Sylred	28.03.	30.03.	04.04.	7,3	26.06.	-7
Pinkcot	28.03.	30.03.	04.04.	7,5	28.06.	-5
Kioto	29.03.	31.03.	05.04.	7,5	01.07.	-2
Luiset	29.03.	31.03.	04.04.	6,0	04.07.	+1
Silvercot	28.03.	30.03.	03.04.	6,0	05.07.	+2
Polonais	29.03.	31.03.	04.04.	6,3	07.07.	+4
Rouge de Rivesaltes	28.03.	29.03.	03.04.	6,5	07.07.	+4
Bergarouge	28.03.	30.03.	03.04.	6,3	12.07.	+9
Bergeron	29.03.	31.03.	05.04.	7,0	13.07.	+10
Helena du Roussillon	28.03.	30.03.	03.04.	5,8	15.07.	+12
Hungarian Best (control)	29.03.	31.03.	04.04.	5,5	03.07.	0

Average time of flowering of apricot cultivars was late March and early April. Among studied cultivars small differences in flowering time were recorded. They were bloomed together with the control cultivar or one day before it. All introduced cultivars manifested longer duration of flowering than the control cultivar (‘Hungarian Best’ with 5.5 days). It

ranged from 5.8 days in cultivar ‘Helena du Roussillon’ to 7.5 days in cultivars ‘Pinkcot’ and ‘Kioto’.

Compared to the results of Milatović (2005) obtained at the same location for the ten-year period (1995-2004) duration of flowering was shorter by three days in average. This difference can be explained by higher temperatures during flowering season in the period of study (2009-2012).

Average time of maturing was from 26<sup>th</sup> of June (‘Sylred’) to 15<sup>th</sup> of July (‘Helena du Roussillon’). Compared to the control cultivar (‘Hungarian Best’) time of maturation was from 7 days earlier to 12 days later. Average difference in the date of maturing between the year with the earliest harvest (2012) and the year with the latest harvest (2010) was 8 days, and among cultivars it ranged from 7 to 10 days.

The average yield per tree ranged from 3.2 kg in ‘Luiset’ to 15.6 kg in ‘Sylred’ (Table 2). These data refer to the yield in the period of initial cropping, when the age of the trees was between three and six years. Cultivars ‘Bergeron’, ‘Sylred’ and ‘Pinkcot’ are characterized by precocity and high initial productivity. Compared with the control cultivar significantly higher yields were achieved in five cultivars: ‘Sylred’, ‘Bergeron’, ‘Pinkcot’, ‘Silvercot’, and ‘Bergarouge’.

Table 2. Yield of apricot cultivars (kg per tree)

Cultivar	Years				Average
	2009	2010	2011	2012	
Sylred	3.7	18.9	33.3	6.7	15.6 a*
Pinkcot	3.3	14.9	20.1	6.2	11.1 b
Kioto	1.8	5.5	6.1	4.6	4.5 d
Luiset	0.2	3.7	6.7	2.3	3.2 d
Silvercot	1.1	8.2	23.5	0.5	8.3 bc
Polonais	1.5	5.5	8.1	2.7	4.4 d
Rouge de Rivesaltes	0.8	2.0	7.4	11.4	5.4 cd
Bergarouge	0.8	10.4	8.7	13.2	8.3 bc
Bergeron	5.4	18.5	30.2	7.6	15.4 a
Helena du Roussillon	0.3	4.0	7.2	6.1	4.4 d
Hungarian Best (control)	0.3	2.1	10.8	2.0	3.8 d

\* Mean values followed by the same letter within a column do not differ significantly according to LSD test at P 0.05

In all cultivars the highest yield was obtained in 2011. Cultivar ‘Sylred’ achieved maximum yield of 33.3 kg per tree or 24.6 t per ha. In 2012 most cultivars achieved low yield due to the occurrence of winter frost (-20.7°C on 9 February), and late spring frost (-2.4°C on 10 April) (Milatović et al., 2013). The highest yield in this year achieved cultivars ‘Bergarouge’ (13.2 kg per tree) and ‘Rouge de Rivesaltes’ (11.4 kg per tree), and they can be considered as less susceptible cultivars to frost.

The obtained results of yield are in accordance with the results of Vachon (2002). He studied the productivity of 24 apricot cultivars during six-year period and found variation of average yield from 3 to 20 kg per tree.

The average fruit weight ranged from 40.28 g in ‘Kioto’ to 67.05 g in ‘Silvercot’ (Table 3). Compared to the control cultivar significantly higher fruit weight had four cultivars: ‘Silvercot’, ‘Sylred’, ‘Polonais’, and ‘Bergeron’, while smaller fruit weight had also four cultivars: ‘Kioto’, ‘Luiset’, ‘Helena du Roussillon’, and ‘Begarouge’. In most cultivars the lowest fruit weight was obtained in 2011, when the highest yield was recorded, while the highest fruit weight was obtained in 2012, when the yield was low.

Table 3. Fruit properties of apricot cultivars (average, 2009-2012)

Cultivar	Fruit weight (g)	Stone weight (g)	Stone share (%)	Fruit dimensions (cm)			Shape index
				Length	Width	Thickness	
Sylred	59.10	2.67	4.52	4.69	4.61	4.53	1.05
Pinkcot	50.02	2.75	5.50	4.46	4.31	4.25	1.09
Kioto	40.28	2.39	5.93	4.18	4.08	3.91	1.10
Luiset	41.96	3.25	7.79	4.30	4.29	4.00	1.07
Silvercot	67.05	3.94	5.88	5.30	5.09	4.46	1.24
Polonais	52.09	3.19	6.12	4.62	4.59	4.35	1.07
Rouge de Rivesaltes	46.09	2.92	6.34	4.67	4.44	4.11	1.19
Bergarouge	42.94	3.01	7.01	4.28	4.17	4.05	1.09
Bergeron	51.79	3.43	6.62	4.61	4.45	4.39	1.09
Helena du Roussillon	42.57	3.14	7.38	4.49	4.14	3.90	1.25
Hungarian Best (control)	47.36	3.51	7.41	4.59	4.61	4.36	1.05
LSD 0.05	3.02	0.18	-	0.22	0.22	0.20	-

Stone weight ranged from 2.39 g ('Kioto') to 3.94 g ('Silvercot'), and its share in the fruit weight ranged from 4.52% ('Sylred') to 7.79% ('Luiset'). Cultivars with larger fruit had relatively smaller stone, i.e. better share of flesh.

Dimensions of the fruit were correlated with the fruit weight. Fruit length varied from 4.2 to 5.3 cm, width from 4.1 to 5.1 cm, and thickness from 3.9 to 4.5 cm. Based on the fruit dimensions, the shape index was calculated, whose values ranged from 1.05 in 'Sylred' to 1.25 in 'Helena du Roussillon'.

Results of pomological fruit characteristics are in accordance with the previous findings for some cultivars (Audergon et al., 2006; Milatović et al., 2006; Szalay et al., 2013).

The content of soluble solids in tested apricot cultivars varied from 12.7% in 'Kioto' to 17.4% in 'Polonais' (Table 4). Cultivars of early maturing time ('Sylred', 'Pinkcot', and 'Kioto') had significantly less content of soluble solids than the control ('Hungarian Best').

Table 4. Fruit quality properties of apricot cultivars (average, 2009-2012)

Cultivar	Soluble solids (%)	Total acids (%)	Soluble solids / Total acids	Sensory evaluation (1-5)	
				Appearance	Taste
Sylred	13.6	1.36	10.0	4.7	4.0
Pinkcot	14.0	1.12	12.5	4.2	3.7
Kioto	12.7	1.57	8.1	3.9	3.5
Luiset	14.8	1.36	10.9	3.0	3.5
Silvercot	16.8	1.63	10.3	4.6	3.9
Polonais	17.4	0.88	19.8	3.7	3.9
Rouge de Rivesaltes	15.1	1.51	10.0	3.4	3.3
Bergarouge	17.0	0.90	18.9	3.9	3.9
Bergeron	14.8	1.39	10.7	4.0	4.0
Helena du Roussillon	17.1	0.75	22.8	3.6	3.9
Hungarian Best (control)	16.2	1.42	11.4	3.5	4.1
LSD 0.05	1.8	0.30	-	0.5	0.6

The total acids content varied from 0.75% in ‘Helena du Roussillon’ to 1.63% in ‘Silvercot’. Significantly less content of total acids than the control had three cultivars (‘Helena du Roussillon’, ‘Polonais’, and ‘Bergarouge’).

Ratio between soluble solids content (consisting mostly of sugars) and acids content indicates the sweetness of the fruit. Higher ratio than in the control was found in four cultivars: ‘Helena du Roussillon’, ‘Polonais’, ‘Bergarouge’, and ‘Pinkcot’.

The data on the chemical composition of fruits are in accordance with the previous findings (Badenes et al., 1998; Gurrieri et al., 2001; Ruiz and Egea, 2008).

Most of the introduced cultivars obtained higher scores for fruit appearance than the control (‘Hungarian Best’). Cultivars ‘Sylred’, ‘Silvercot’ and ‘Pinkcot’ particularly stand out for attractive fruit appearance. Taste of all introduced cultivars was evaluated with lower scores than the control.

### Conclusion

Based on the four-year evaluation of 10 French apricot cultivars in the Belgrade area, the best results have shown by ‘Sylred’, ‘Silvercot’ and ‘Pinkcot’, which can be recommended for growing, predominantly for fresh consumption. In addition, ‘Bergeron’ and ‘Bergarouge’ can also be recommended as cultivars of combined traits, both for fresh consumption and processing.

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## EFFECTS OF FOLIAR FERTILIZERS ON MAIZE INBRED LINES

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### Abstract

Crop fertilization is one of the most important cropping practises. Fertilisation provides optimal plant growth and development. Foliar fertilizing enables rapid absorption and quick plant response to applied fertilizer. Foliar fertilizing also can contain microelements and amino acid. The aim of this study was to examine effect of two foliar fertilizers (formulation: 12:4:6+0.2MgO+ME+AA and 10:40:10+4MgO+ME) on five maize inbred lines. The first fertilizer was applied at the 5-6 leaf stage of maize, while second in 11-12 leaf stage. Influence of foliar fertilizers was examined on fresh matter at 48h, 21 day after application and in flowering stages and on grain yield. Application of foliar fertilizers increase fresh matter in maize lines in all examined stages compared to control. The greatest differences in fresh matter were recorded for 21 days after application of foliar fertilizers compared to control. Foliar fertilizers also increased grain yield of maize lines. Although both fertilizers significantly increased fresh matter and grain yield, the first fertilizer (12:4:6+0.2MgO+ME+AA) shows as better than the other tested fertilizer.

**Key words:** maize inbred lines, foliar fertilizers

### Introduction

Fertilization is one of the most important cropping practices that provide optimal plant growth and development. Fertilizing could be classified as basic and foliar fertilizing. Genetic potential is closely linked with fertilizing. Optimal nutrition also implies better condition and contributes to greater resistance to abiotic and biotic stress, first of all to drought, diseases and pests (Varallyay, 2006). Plant response to foliar fertilisation is quick due to rapid absorption of elements (Oosterhuis, D., 2009). Foliar fertilizers generally include microelements and amino acids that have positive effect on plants. In seed maize crop herbicide application is unavoidable practice, mixed application of herbicide and foliar fertilizers could lead to diminishing of herbicide stress (Brankov et al., 2011.). An additional impact of foliar N application is reflected through better maize yields (Abdel-Hamid et al., 2006; Shirvani Sarakhsi et al., 2010.). On the other hand, fertilisers incorporated into basic formulations are subjected to leaching, impact of microorganisms, etc., when they become inaccessible to plants (Lopez-Bellido et al., 2006). Dragi eviC et al. (2010) also stated that foliar fertilising affects the increase of plants fresh matter and therefore it positively affects the plant growth.

The objective of the present study was to test effects of two formulations of foliar fertilisers applied on fresh matter and grain yield of five maize inbred lines.

### Material and methods

Field experiment was set up at slightly calcareous chernozem in the experimental field of the Maize Research Institute, “Zemun Polje”, during 2010 and 2011, with wheat as a preceding crop in both years. The aim of experiment was to test effects of two foliar fertilizers on five maize inbred lines by measuring fresh matter and grain yield. Foliar fertilizers were applied in different phase of maize growth: foliar fertilizer F1 - Activeg (12:4:6+0.2MgO+ME+AK) was applied at 5-6 leaf stage, while F2 Soluveg Green (10:40:10+4MgO+ME) in 10-12 leaf stage of maize. The four-replicate trail was set up according to the split-plot arrangement. The elementary plot size was 16.8m<sup>2</sup>, while the plant density amounted to 60,000 plants ha<sup>-1</sup>. Maize inbred lines were sown manually on April 26 and 27 in both years. Samples of four plants for measuring plant fresh matter were taken 48 hours and 21 days after foliar fertilizers were applied. In flowering stage fresh matter of maize leaves were measured. Maize grain yield was measured after harvesting and was calculated at 14% moisture. Obtained data were statistically processed by ANOVA and differences between means were tested by the least significant difference (LSD<sub>0.05</sub>). Meteorological data collected during the trail duration are presented in table 1.

Table 1. Precipitation and average air temperatures for the period April-October 2010/11

Months	Precipitation (mm)		Temperatures (°C)	
	2010	2011	2010	2011
April	44.0	14.9	13.2	13.4
May	64.1	89.6	17.5	16.8
June	167.3	26.2	21.0	21.5
July	35.6	44.0	23.2	23.3
August	68.2	66.0	23.1	23.9
September	68.0	32.6	17.6	21.6
Average	447.2	273.3	19.3	20.1

### Results and discussion

First recorded differences in treatments were recorded 48 after foliar fertilizers application. All lines, except L3, had higher values of fresh matter comparing to the control treatment in first foliar fertilizer (F1). In average there was an increasing of 25.7% on F1 treatment than in control. On the other hand, second foliar fertilizer (F2) increased average fresh matter 40.3% comparing to control. Due to late application of F2 there are higher values of estimated fresh matter. It is interesting to emphasize that L3 on F2 had the best response to fertilizing (increasing of fresh matter of 57.2% comparing to control) (Table 2).

Similar results were recorded at second measurement, 21 days after foliar fertilization application (Table 3). Both fertilizers statistically increased fresh matter of maize inbred lines. First foliar fertilizer increased average values of fresh matter 16.1%, while second (F2) for 13.9%.

Table 2. Effects of foliar fertilizers on fresh matter of maize inbred lines 48h after foliar fertilizer application, (g plants<sup>-1</sup>) average 2010/11.

	Fresh matter of plants (g plant <sup>-1</sup> )					Average
	L1	L2	L3	L4	L5	
Control	10.9	5.5	13.1	11.7	5.6	9.36 b
F1	15.2	8.0	13.1	15.9	10.7	12.6 a
Average	12.8	6.7	13.1	13.8	8.1	
Control	112.6	127.7	142.4	136.5	56.5	115.1
F2	210.5	150.4	332.9	162.5	108.4	192.9
Average	161.5	139.0	237.6	149.5	82.4	

Table 3. Effects of foliar fertilizers on fresh matter of maize inbred lines 21 days after foliar fertilizer application, (g plants<sup>-1</sup>) average 2010/11.

	Fresh matter of plants (g plant <sup>-1</sup> )					Average
	L1	L2	L3	L4	L5	
Control	112.6	127.7	142.4	122.3	56.5	112.3 b
F1	162.1	133.9	147.8	160.3	65.2	133.9 a
Average	137.3	130.8	145.1	141.3	60.8	
Control	209.4	275.9	342.3	315.4	267.7	282.1 b
F2	271.8	300.9	403.8	382.4	279.9	327.8 a
Average						

According to obtained results first foliar fertilizer significantly increased fresh matter of maize leaves (Table 4), while second fertilizer was not record increased values of fresh matter. Lines L1 and L5 showed the highest increase of fresh matter (6.6% and 11.6%). The fresh matter also varied among genotypes, but considering their belonging to various maturity groups, such results had been expected.

Foliar fertiliser did not only affect the fresh matter of maize inbreds, but also induced the yield increase (Table 5). Grain yield was higher in both treatments with foliar fertilizer comparing to control. Similarly, Dragi eviC et al. (2010) and Brankov et al. (2011) stated that the application of foliar fertiliser increased maize grain yield. The highest average yield in all five maize inbreds was measured in the treatment F1 (increasing of 15.2% comparing to control), which had been applied at the 5-6-leaf stage, while a somewhat lower yield was registered in the F2 treatment (increasing of 11.4% comparing to control). The grain yield of L2 and L3 expressed the lowest variation under the influence of applied fertilisers in relation to the control. Statistical analyses indicated significantly differences between treatments with foliar fertilizers and control.

Table 4. Effects of foliar fertilizers on fresh matter of maize inbred lines at flowering stage, (g leaf<sup>-1</sup>) average 2010/11.

	Fresh matter of leaves (g plant <sup>-1</sup> )					Average
	L1	L2	L3	L4	L5	
Control	162.1	254.6	301.3	360.1	290.6	273.7 b
F1	173.6	250.4	316.1	372.1	328.9	288.2 a
F2	163.1	254.6	301.3	360.1	290.6	273.9 b
Average	166.3	253.2	306.2	364.1	303.4	

Table 5. Maize grain yield, average 2010/11.

	Grain yield (t ha <sup>-1</sup> )					Average
	L1	L2	L3	L4	L5	
Control	1.86	3.11	6.09	5.6	2.81	3.9 b
F1	2.57	3.26	6.28	6.43	4.39	4.6 a
F2	1.97	3.19	6.21	5.6	5.03	4.4 a
Average	2.13	3.19	6.19	5.88	4.08	

### Conclusion

Based on obtained results it can be concluded that the application of foliar fertilisers significantly increased fresh matter of maize inbred lines. Foliar fertilizing enables fast and rapid plant response and could provide better growth condition. As a result of foliar fertilising plants of maize lines had higher total fresh matter, and grain yield comparing to control. However, the treatment with the formulation F1 expressed better results regarding measured parameters than the treatment with the formulation F2.

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## CHARACTERIZATION OF NATURAL MEADOWS AND PASTURES IN PEŠTER

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### Abstract

The phytocenological composition estimated on grasslands of hilly-mountain area of Pešter (vicinity of Sjenica). Meadows and pastures in this area developed on forest land based on stone with feldspat and silicate. On the geological base of nonorganic sediments, sandstone, gravels and clays and seldom phillites are developed different associations. In the investigation identified 124 different plant species which belong to different plant life form and different associations. Investigation carried out from May to August in 2012 year. In this region prevail natural pasturelands distributed on large plateau approximately 1200m above the sea level on the acid soil type pseudogleys, luvisol. Large area used for grazing and meadow area near the villages exploited for hay production and after that used for grazing. These natural grasslands exist without application of scientific farming measure of technology. In these successive inventories of lands registered floristic associations and identified individual plant species. Investigation showed different floristic elements and phytocenological associations on meadow and pastures.

**Key words:** Floristic diversity, meadow, pasture, mountain

### Introduction

Meadows and pastures developed mainly on the lands after deforestation and spread in different geographic, ecological and orographic condition on different type of soil. All these factor connected to floristic and phytocenological diversity. Numerous plant species on natural meadows and pasture are desirable for animal feed. Some of plants can be harmful for animals. Pastures are used for grazing during spring, summer and autumn season, while meadows hay produced and after that can be used for grazing. Meadows usually situated near the villages integrated within arable land, while pastures spread at the higher altitudes on the larger area. Except climatic factors, orographic exposition, rocky substrate and soil fertility, the relation of man to nature protection is important for survival plant diversity. Meadows and pastures are rich with floristic and phytocenological diversity (Koji et al., 2005). The plant communities on meadow and pasture differ depending of parent rock that underlies soil where are developed and can classified on: basic rocks with feldspat and ultra basic rocks; communities on limestone; communities on acid siliceous rocks; hilly and mountainous communities of wide ecological amplitude; alpine communities. The statement of meadows and pastures depends on position, environmental conditions and how they are managed. The natural grasslands have less productivity of biomass that is under the high environmental influence. The grasslands production is very important sustainable source of animal feed and production of milk and meat. The intensive study of grasslands reported that different ecological conditions have influence to developing different meadow associations which

differ according to their floristic elements and their productivity association (Lazarevi et al., 2004). These authors noted that on hilly-mountainous region of Serbia, the greatest presence and economical importance have associations *Danthonietum calycinae*, *Festucetum rubrae*, *Agrostietum vulgaris* and *Nardetum strictae* (Lazarevi et al., 2009) and that productivity increase under anthropogenic influence.

The objective of this study was analysis of floristic and phytocenological characteristics on meadows and pastures in Pešter plateau.

### Material and methods

Investigation of phytocenological diversity carried out on meadows and pasture in Pešter plateau. Study of phytocenological associations conducted according to methods of the French-Swiss school Braun-Blanquet (Braun-Blanquet, 1928, 1921) that is, in Serbia, widely accepted in phytocenological researches. The floristic and phytocenological estimation carried out on surface of 50 m<sup>2</sup>, in 4 sites in location Duga Poljana and Štavalj. Estimation of floristic diversity by records in 23. June, 30. June, 10. July and 20. July 2012. The analyzed meadows and pastures developed on soil type pseudogleys and luvisol. The average pH value was 4.2. In the soil content were found humus (4.5-6.0%), Nitrogen (0.2%), low content of Phosphorus (1.8 mg per 100g of soil) and Potassium in average 19.2 mg per 100 g of soil). The standard laboratory methods were used for the determination of the soil quality.

### Results and discussion

Floristic elements identified in four location on Pešter plateau, developed under the specific environmental condition (frequently changes values of temperature, humidity, sun-light regime and precipitation). Analyzed locations situated at eastern, southeastern, southwestern and northeastern expositions.

On analyzed location registered 124 plant species, including: 118 herbaceous plants, 4 shrubs and 2 tree species (Table 1.). Identified plants belong to different group of floral elements Euro-Asian (cosmopolite plants), middle European (mesophilic plants), sub-Mediterranean (xerophilic plants) and Balkan-Appennine floristic elements (xerophilic). Investigated meadows and pastures are very rich and diverse in the floristic and phytocenological composition. Identified floristic elements different phytocenological associations: *Ass. Danthonietum calycinae*, *Ass. Festuco-Chrysopogonetum grylli*, etc. *Ass. Festucetum vallesiaca*, *Ass. Festucetum fallaci*, *Ass. Cynosuretum cristati*, *Ass. Cariceto-Brometum erecti*.

These associations on warmer exposures are represents grasslands on serpentine and meadows which are mostly developed in the oak-pine-belt trees, ranging in altitude from 1000 m and higher. In this area most of the sward was occupied by meadows for their ecological and floristic elements belong to the plant community composition of grass species *Danthonietum calycinae*; *Cynosurus cristatus*, *Bromus racemosus*, *Festuca vallesiaca*, *Festuca rubra*, broadly.

Similar association on mountain region of Serbia (Stara planina) registered (Tomi et al., 2003). The wealth of floristic elements of ruderal association developed on soils which are typical for mountainous area, also registered in other region example Bosnia and Herzegovina (Petroni and Pavlovi, 2006). Areal spectrum of *Cirsietum candelabri* association points to the presence different floral elements: Euro-Asian, boreal, cosmopolitan etc.

Table 1. Floristic elements on Pester Plateau (Duga Poljana and Štavalj)

Species	1	2	3	4		1	2	3	4
<i>Campanula trachelium</i> L.	+1		+1	+1	<i>Briza media</i> L.		+1.1		+1.1
<i>Campanula persicifolia</i> L.	+1	+1	+1	+1	<i>Campanula patula</i> L.		+1	+1	
<i>Anemone nemorosa</i> L.	+1.1	+1.1	+2.2		<i>Carex montana</i> L.	+1		+1	
<i>Avena pubescens</i> L.		+1.1	+1.1		<i>Cerastium brachypetalum</i> L.		+1		+1
<i>Alchemilla vulgaris</i> L.	+1	+1.1	+1.1		<i>Chamaecytisus supinus</i> L.	+1			
<i>Cephalantrea longipholia</i> L.	+1	+1	+1		<i>Cytisus scoparius</i> L.		+2.2		+1
<i>Cephalantrea rubra</i> L.		+1	+1		<i>Bromus racemosus</i> L.	+1	+1	+1	
<i>Centaruea jacea</i> L.		+1	+1	+1	<i>Dianthus armeria</i> L.			+1	
<i>Centaurea montana</i> L.				+1	<i>Digitalis ferruginea</i> L.		+1		
<i>Geranium sanguineum</i> L.	+1	+1	+1		<i>Dorycnium herbaceum</i> L.				+1
<i>Trifolium alpestre</i> L.		+1.1		+1.1	<i>Festuca vallesiaca</i> L.	+1.1			
<i>Veratrum album</i> L.			+1	+1	<i>Galium verum</i> L.				+1
<i>Solidago virga-aurea</i> L.			+1	+1	<i>Hypericum montanum</i> L.	+1			
<i>Bromus erectus</i> L.	+1	+1	+1		<i>Lathyrus niger</i> L.				+1.1
<i>Aremonia agrimonioides</i> L.	+1.1		+1		<i>Leontodon hispidus</i> L.				+1
<i>Brachypodium sylvaticum</i> L.		+1.1		+1.2	<i>Leucanthemum vulgare</i> L.		+1.1		+1.1
<i>Filipendula hexapetala</i> L.	+1	+1	+1.1		<i>Lolium perenne</i> L.		+1.1		+1.1
<i>Galium boreale</i> L.		+1	+1	+1	<i>Linum hirsutum</i>			+1.1	
<i>Galium mollugo</i> L.	+1	+1	+1	+1	<i>Luzula campestris</i> L.				+1
<i>Prunella vulgaris</i> L.	+1	+1.1	+1	+1	<i>Luzula luzuloides</i> L.				+2.2
<i>Astrantia major</i> L.	+1	+1	+1	+1	<i>Luzula pilosa</i> L.	+1			
<i>Phyteuma spicatum</i> L.		+1	+1		<i>Lysimachia vulgaris</i> L.	+1.1			+1
<i>Cynosurus cristatus</i> L.			+1.1	+1	<i>Melampyrum pratense</i> L.			+1.1	
<i>Hypericum perforatum</i> L.			+1.1	+1	<i>Melica nutans</i> L.	+1	+1.1		
<i>Fragaria vesca</i> L.	+1	+1	+1.1		<i>Melittis melissophyllum</i> L.	+1.1			
<i>Lilium martagon</i> L.			+1	+1	<i>Mercurialis perennis</i> L.		+1		
<i>Lilium hirsutum</i> L.			+1	+1	<i>Myosotis arvensis</i> L.				+1
<i>Trifolium repens</i> L.	+1	+1	+1	+1	<i>Plantago altissima</i> L.		+1.1	+1	
<i>Ajuga reptans</i> L.	+1	+1	+1	+1	<i>Plantago argentea</i> L.	+1			
<i>Dactylis glomerata</i> L.	+1	+1.1	+1.1	+1.1	<i>Plantago major</i> L.	+1		+1.1	
<i>Trifolium montanum</i> L.	+1	+2.2	+1.1	+1.1	<i>Poa nemoralis</i> L.		+1.1	+1	
<i>Viola silvestris</i> L.	+1	+1	+1	+1	<i>Poa pratensis</i> L.		+1.1		+1.1
<i>Aegopodium podagraria</i> L.	+1			+1	<i>Polygala comosa</i> L.				+1.1
<i>Carex praecox</i> L.			+1	+1.1	<i>Polystichum lobatum</i> L.	+1			
<i>Cirsium acaule</i> L.		+1	+1.1		<i>Potentilla erecta</i> L.	+1	+1		
<i>Coronilla varia</i> L.	+1	+1			<i>Potentilla australis</i> L.		+2	+1	
<i>Crepis viscidula</i> L.	+1			+1	<i>Potentilla heptaphylla</i> L.				+1
<i>Danaa cornubiensis</i> L.	+1	+1	+1		<i>Ranunculus repens</i> L.		+1	+1	
<i>Danthonia provincialis</i> L.			+1.1	+1.2	<i>Primula vulgaris</i> L.	+1			+1
<i>Deschampsia flexuosa</i> L.		+1		+1.2	<i>Pteridium aquilinum</i> L.		+1		
<i>Dorycnium herbaceum</i> L.	+1		+2		<i>Pulmonaria officinalis</i> L.	+1			+1
<i>Euphorbia amygdaloides</i> L.	+1			+1	<i>Rumex acetosa</i> L.		+1		+1.1
<i>Festuca heterophylla</i> L.		+1.1		+1.1	<i>Selinum carvifolia</i> L.	+1			
<i>Galium silvaticum</i> L.	+1			+1.1	<i>Silene italica</i> L.		+1		
<i>Galium cruciata</i> L.	+1				<i>Stelaria graminea</i> L.	+2			
<i>Galium rubioides</i> L.			+1		<i>Thymus longicaulis</i> L.			+1	
<i>Geum rivale</i> L.		+1			<i>Thymus serpyllum</i> L.			+2	
<i>Helleborus odoratus</i> L.					<i>Trifolium hybridum</i> L.		+1		+1.1
<i>Hieracium bauhinii</i> L.		+1.1		+1.1	<i>Trifolium medium</i> L.	+1.1			+1.1
<i>Hieracium cymosum</i> L.	+1			+1	<i>Trifolium pratense</i> L.	+1.1		+1.1	
<i>Hieracium maculatum</i> L.	+1	+1			<i>Festuca rubra</i> L.		+1.1	+1.1	
<i>Hieracium panosum</i> L.	+1		+1		<i>Verbascum lanatum</i> L.	+1			+2
<i>Hieracium pilosella</i> L.		+1		+1.1	<i>Veronica paecox</i> L.		+1	+1	
<i>Inula hirta</i> L.		+1.1		+1.1	<i>Vicia cassubica</i> L.	+1		+1	
<i>Inula salicina</i> L.	+1		+1		<i>Vicia cracca</i> L.			+1.1	+1.1
<i>Lamium galeobdolon</i> L.			+1	+1	<b>Shrubs</b>				
<i>Lathyrus venetus</i> L.		+1	+1	+1	<i>Genista ovata</i> L.	+1			+1.2
<i>Leontodon hispidus</i> L.	+1			+1	<i>Teucrium chamaedrys</i> L.	+1.1			
<i>Polygonatum odoratum</i> L.	+1				<i>Prunus spinosa</i>		+1		+1
<i>Silene nutans</i> L.		+1		+1.1	<i>Rubus canescens</i>		+1	+1	
<i>Stachys officinalis</i> L.		+1.1	+1	+1.1	<b>Trees</b>				



<i>Stelaria holostea</i> L.	+1.1	<i>Pyrus pyraaster</i>	+1.1	+1
<i>Veronica officinalis</i> L.	+1	<i>Quercus cerris</i>	+1.1	+1

The ordinal number of the record 1, 2, 3, 4 ; Date of the record 23.06.'2012, 30.06.'12; 10.07.'2012, 20.07.'2012;  
 Location: Duga Polajna, Štavalj; Surface record (50m<sup>2</sup>) 200; Exposition E S-SE, S-SW, N-NE,  
 Geological base Phillites Sandstones, clays, hornstones and marls; Soil type: luvisol, pseudogley.

These floral elements in the area of oak-pine forests that span the slopes, in natural meadows, forest clearings and plains, mainly in shallow, rocky soil (limestone, silica substrates and acidic soil). Similar results on mountain region reported Koji et al., 2005; Tomi et al., 2005.

The identified herbaceous species cover soil in range up to 90%. The most dominant species are: *Campanula persicifolia*, *Prunella vulgaris*, *Ajuga reptans*, *Dactylis glomerata*, *Trifolium montanum*. Also, frequently present plant species but with reduced sociability are: *Viola silvestris*, *Aegopodium podagraria*, *Anemone nemorosa*, *Aremonia agrimonioides*, *Brachypodium silvaticum*, *Cirsium acaule*, *Danaa cornubiensis*, *Euphorbia amygdaloides*, *Festuca heterophylla*, *Galium cruciata*, *Galium silvaticum*, *Genista ovata*, *Geranium sanguineum*, *Helleborus odoratus*, *Helianthemum nummularium*, *Hieracium baehiniae*, etc.

On the investigated locations meadow and pastures used for grazing during long-term period. The meadows were grown and lasted in period from May to July. However, anthropogenic factor can influence to make system of grazing and improve grassland production. Developing grazing system can contribute to improving production of forage for livestock (Lazarević et al., 2009). Also, system of seasonal grazing animals on a particular area, allows the land than not used for grazing to rest and growing new forage. Most farmers try rotational grazing because of economic factors. Grazing may stimulate pasture growth, decrease soil erosion potential, minimal pesticides and fertilizer application.

### Conclusions

The most changes of sustainable meadows and pastures in this area threatened by land abandonment, afforestation, overgrazing and rarely by using land for arable farming other crops and urban development. Floristic composition contains 124 species: 118 herbaceous species, 4 shrub species and 2 tree species. Identified floral elements present mixture of different communities of plants with identified species floral elements Euro-Asian (cosmopolite plants), middle European (mesophilic plants), sub-Mediterranean (xerophilic plants) and Balkan-Apennine floristic elements (xerophilic). The floristic wealth developed on deforested surfaces on soil pseudogley and luvisol. The geological base is nonorganic sediments, sandstone, gravels and clays and rarely phillites.

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## WEED SPECIES AND THEIR MANAGEMENT IN ORGANIC SWEET CHERRY PRODUCTION IN ISPARTA (EGIRDİR) PROVINCE

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### Abstract

This study was conducted in 2003, 2004 and 2005 in Isparta (Egirdir) province in Turkey. Organic agriculture and IPM (Integrated Pest Management) implementations were applied in sweet cherry orchards were determined main weed species and density of annual and perennial weeds. Control methods were applied against weeds. The studies on IPM orchard were carried out for compare with organic orchard. Based on the results of surveys, *Sorghum halepense*, *Cuscuta campestris*, *Lactuca serriola*, *Trifolium* sp., *Polygonum convolvulus*, *Cynanchum acutum*, *Cirsium arvense* and *Veronica hederifolia*. *Sorghum halepense*, *Cuscuta campestris*, *Lactuca serriola* and *Trifolium* sp. were the most frequently observed weeds species in organic orchard. *Cynodon dactylon*, *Convolvulus arvensis*, and *Cynanchum acutum* were the most frequently determined weed species in IPM orchard.

In organic and IPM orchard was found perennial weeds that they were hard to control. For the control of perennial weeds were applied deep tillage and their roots removed from soil. Annual weeds were controlled by hand picking and tillage. Although in organic orchard were applied tillage to control perennial weeds this application were not sufficient and were also applied mowing twice and digging in some years. These were enough to control weeds in organic cherry orchard. Same cultural implementations were done to control weeds in IPM orchard. Any herbicide was not applied for control of weed in IPM orchard, because sweet cherry trees were under 4 years.

**Keywords:** Egirdir, sweet cherry, weed, organic, control

### Introduction

Cherry [(*Prunus avium*) (Rosales: Rosaceae)] is one of the important fruit crops of Turkey. It has an important position in the Turkish economy so that it matures in early seasons and its production for domestic market and especially as an export product. According to 2012 statistics there are 24.170.000 cherry trees in Turkey and Turkey is the first of the world by producing 470.887 tonnes cherry (Anonymous, 2012).

Turkey has been producing organic agricultural products since the mid 1980s. Organic agricultural applications are daily in Turkey and in the world as a solution for the diminishing of environmental pollution and human health problems caused by conventional agriculture. Cherry orchards have various diseases insects and weeds. Weeds may be also infested with a variety of annual and perennial weeds, each competing with the trees for water and nutrients. Competition for these resources is of greater concern with newly planted trees because weeds can reduce their growth, vigor, and delay production. Cherry orchards have various diseases insects and weeds. Weeds may be also infested with a variety of annual and perennial weeds, each competing with the trees for water and nutrients. Competition for these resources is of greater concern with newly planted trees because weeds can reduce their growth, vigor, and delay production.

### Materials and methods

Main material in the research is the weeds of organic and IPM sweet cherry orchards. Aiding materials were a orchard with early, mid season and late cherry varieties. This study was conducted to determine density of weeds species in Organic and Integrated Pest Management (IPM) orchard of cherry in Isparta (Egirdir) province. Varieties of cherries in the orchard that were used Early Burlat, Napoleon and Ziraat 0900 varieties. Survey was carried out in april and july in 2003-2004 and 2005. The random sampling was made according to representing at least 2% of the orchards of cherry . 1/4 m<sup>2</sup> were taken in a sampling area at least 20 times. The weeds within the frame were counted and density of weeds were determined. The identification of weeds was made according to the references of Davis (1965–1988), Baytop (1989), Ulu et al. (1993), Güner et al. (2000).

### Results and discussion

Fifty-eight weed species and Fifty-one genus belong to twenty- three families were identified in the organic and IPM orchard of cherry surveyed. The density of the weed species in organic and IPM orchard of cherry in Isparta (E irdir) province are given in Table 1 and Table 2.

Table 1. Weed species and weed density in organic cherry orchards in Isparta (E irdir) province in 2003, 2004 and 2005.

Date	Weed Species	Weed Density Plants/m <sup>2</sup>
27.4.2003	<i>Urtica urens</i> L.	1.4
	<i>Melilotus officinalis</i> (L.)Desr.	1.2
	<i>Convolvulus arvensis</i> L.	3.9
	<i>Geranium pusillum</i> Burn.	0.7
	<i>Mentha longifolia</i> (L) Huds.	0.8
	<i>Galium aparine</i> L.	0.7
	<i>Plantago lanceolata</i> L.	1.2
	<i>Artemisia</i> sp.	0.9
	<i>Euphorbia stricta</i> L.	1.3
	<i>Stachys</i> sp.	4.7
	<i>Daucus carota</i> L.	0.6
	<i>Cynanchum acutum</i> L.	4.6
	<i>Plantago major</i> L.	1.7
	<i>Sorghum halepense</i> (L.) Pers.	4.7
<i>Anthemis cotula</i> L.	0.6	
29.4.2004	<i>Alopecurus myosuroides</i> Hudson	4.7
	<i>Artemisia vulgaris</i> L.	0.8
	<i>Capsella bursa-pastoris</i> (L.) Medik.	0.5
	<i>Cerastium dichotomum</i> L.	1.7
	<i>Chenopodium album</i> L.	1.7
	<i>Cirsium arvense</i> (L.) Scop	6.2
	<i>Convolvulus arvensis</i> L.	4.0

	<i>Cynodon dactylon</i> (L.) Pers.	0.5
	<i>Cynanchum acutum</i> L.	4.5
	<i>Lactuca serriola</i> L.	2.8
	<i>Plantago lanceolata</i> L.	1.1
	<i>Plantago major</i> L.	0.5
	<i>Ranunculus arvensis</i> L.	1.1
	<i>Raphanus raphanistrum</i> L.	6.2
	<i>Senecio vernalis</i> Waldst.&Kit.	1.1
	<i>Sonchus asper</i> (L.) Hill	0.5
	<i>Veronica hederifolia</i> L.	6.8
	<i>Xanthium strumarium</i> L.	4.5
12.7.2004	<i>Acroptilon repens</i> (L.) DC.	0.8
	<i>Amaranthus retroflexus</i> L.	0.4
	<i>Chenopodium album</i> L.	4.0
	<i>Chondrilla juncea</i> L.	0.4
	<i>Cirsium arvense</i> (L.) Scop	7.6
	<i>Convolvulus arvensis</i> L.	6.2
	<i>Cynanchum acutum</i> L.	8.0
	<i>Cynodon dactylon</i> (L.) Pers.	4.0
	<i>Lactuca serriola</i> L.	0.8
	<i>Polygonum convolvulus</i> L.	1.2
	<i>Raphanus raphanistrum</i> L.	0.4
	<i>Sorghum halepense</i> (L.) Pers.	4.8
	<i>Xanthium strumarium</i> L.	0.4
18.07.2005	<i>Acroptilon repens</i> (L.) DC.	0.8
	<i>Amaranthus retroflexus</i> L.	0.4
	<i>Artemisia vulgaris</i> L.	6.2
	<i>Bromus</i> sp.	1.1
	<i>Chenopodium album</i> L.	4.0
	<i>Cirsium arvense</i> (L.) Scop	0.4
	<i>Convolvulus arvensis</i> L.	1,6
	<i>Cuscuta campestris</i> Yuncker	9.6
	<i>Cynanchum acutum</i> L.	2.0
	<i>Cynodon dactylon</i> (L.) Pers.	7.3
	<i>Lactuca serriola</i> L.	9.3
	<i>Lolium temulentum</i> L.	0.6
	<i>Plantago major</i> L.	0.9
	<i>Polygonum convolvulus</i> L.	8.0
	<i>Sorghum halepense</i> (L.) Pers.	9.8
	<i>Trifolium</i> sp.	9.1
	<i>Xanthium strumarium</i> L.	1.3

Table 2. Weed species and weed density in IPM cherry orchards in Isparta (E irdir) province in 2003, 2004 and 2005.

Date	Weed Species	Weed Density Plants/m <sup>2</sup>
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27.04.2003	<i>Urtica urens</i> L.	1.4
	<i>Melilotus officinalis</i> (L.)Desr.	1.2
	<i>Convolvulus arvensis</i> L.	3.9
	<i>Geranium pusillum</i> Burn.	0.7
	<i>Mentha longifolia</i> (L) Huds.	0.8
	<i>Galium aparine</i> L.	0.7
	<i>Plantago lanceolata</i> L.	1.2
	<i>Artemisia</i> sp.	0.9
	<i>Euphorbia stricta</i> L.	1.3
	<i>Stachys</i> sp.	4.7
	<i>Daucus carota</i> L.	0.6
	<i>Cynanchum acutum</i> L.	4.6
	<i>Plantago major</i> L.	1.7
	<i>Sorghum halepense</i> (L.) Pers.	4.7
	<i>Anthemis cotula</i> L.	0.6
29.4.2004	<i>Alopecurus myosuroides</i> Hudson	4.5
	<i>Acroptilon repens</i> (L.) DC.	2.2
	<i>Adonis flammea</i> Jacq.	1.1
	<i>Bromus sterilis</i> L.	0.5
	<i>Cardaria draba</i> (L.) Desv.	1.7
	<i>Chenopodium album</i> L.	5.1
	<i>Cirsium arvense</i> (L.) Scop.	1.7
	<i>Convolvulus arvensis</i> L.	9.8
	<i>Cynanchum acutum</i> L.	9.7
	<i>Cynodon dactylon</i> (L.) Pers.	9.2
	<i>Geranium</i> sp.	0.5
	<i>Lamium amplexicaule</i> L.	3.5
	<i>Lactuca serriola</i> L.	2.2
	<i>Malva neglecta</i> Wallr.	0.5
	<i>Polygonum convolvulus</i> L.	0.5
	<i>Ranunculus arvensis</i> L.	1.7
	<i>Rumex crispus</i> L.	0.5
	<i>Sinapis arvensis</i> L.	2.2
	<i>Veronica hederifolia</i> L.	2.2
<i>Veronica triphyllos</i> L.	1.1	
12.7.2004	<i>Amaranthus retroflexus</i> L.	5.6
	<i>Artemisia vulgaris</i> L.	0.4
	<i>Chenopodium album</i> L.	2.4
	<i>Cirsium arvense</i> (L.) Scop.	1.6
	<i>Convolvulus arvensis</i> L.	9.9
	<i>Crepis foetida</i> L.	0.8
	<i>Cynodon dactylon</i> (L.) Pers.	13.2
	<i>Cynanchum acutum</i> L.	7.6
	<i>Datura stramonium</i> L.	0.4
	<i>Heliotropium europaeum</i> L.	0.4
	<i>Hyoscyamus niger</i> L.	1.6

	<i>Lactuca serriola</i> L.	1.6
	<i>Plantago major</i> L.	0.4
	<i>Polygonum convolvulus</i> L.	0.8
	<i>Raphanus raphanistrum</i> L.	0.4
	<i>Setaria glauca</i> (L.) P. Beauv.	0.4
	<i>Sorghum halepense</i> (L.) Pers.	4.4
	<i>Xanthium strumarium</i> L.	5.2
18.07.2005	<i>Acroptilon repens</i> (L.) DC.	1.1
	<i>Amaranthus retroflexus</i> L.	3.4
	<i>Anthemis</i> sp.	0.6
	<i>Chenopodium album</i> L.	2.4
	<i>Cirsium arvense</i> (L.) Scop.	1.9
	<i>Convolvulus arvensis</i> L.	10.1
	<i>Cynodon dactylon</i> (L.) Pers.	12.4
	<i>Cynanchum acutum</i> L.	6.5
	<i>Galium</i> sp.	0.4
	<i>Heliotropium europaeum</i> L.	0.5
	<i>Hyoscyamus niger</i> L.	1.2
	<i>Lactuca serriola</i> L.	2.3
	<i>Plantago major</i> L.	0.3
	<i>Polygonum convolvulus</i> L.	0.7
	<i>Raphanus raphanistrum</i> L.	0.2
	<i>Rumex</i> sp.	1.1
	<i>Sorghum halepense</i> (L.) Pers.	5.1
	<i>Xanthium strumarium</i> L.	3.7
	<i>Vicia</i> sp.	0.4

Based on the results of surveys (Table 1), *Sorghum halepense*, *Cuscuta campestris*, *Lactuca serriola* and *Trifolium* sp. were the most common observed weeds species in organic orchard. *Cynodon dactylon*, *Convolvulus arvensis*, and *Cynanchum acutum* were the most frequently determined weed species in IPM orchard (Table 2).

### Conclusion

In organic and IPM orchard was found perennial weeds that they were hard to control. For the control of perennial weeds were applied deep tillage and their roots removed from soil. Annual weeds were controlled by hand picking and tillage. Although in organic orchard were applied tillage to control perennial weeds this application were not sufficient and were also applied mowing twice and digging in some years. These were enough to control weeds in organic cherry orchard. Same cultural implementations were done to control weeds in IPM orchard. Any herbicide was not applied for control of weed in IPM orchard, because sweet cherry trees were under 4 years.

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**COMPOSTED POSIDONIA, CHICKEN MANURE AND OLIVE MILL RESIDUES,  
AN ALTERNATIVE TO PEAT AS SEED GERMINATION AND SEEDLING  
GROWING MEDIA IN TUNISIAN NURSERY?**

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**Abstract**

In order to reduce the peat use in Tunisian nurseries, we tested new types of composts for seed germination and seedling growth of tomato. These composts were made at three different combinations from Posidonia (*Posidonia oceanica*), chicken manure and solid fraction of olive mill residues. These wastes are abundant and therefore considered as a pollution source in Tunisia. Tomato seeds were sown in potting media containing mixtures of three composts (C<sub>1</sub>, C<sub>2</sub> and C<sub>3</sub>) and peat at increasing ratios (10%, 30% and 50% v/v). Control potting media consisted of using 100% peat. Weekly and during five weeks the percentage seed germination and the seedling length were studied. At the end of the experiment, shoot and root dry matter weights were measured. Toxicity test of compost extracts was conducted on tomato and radish seeds. The results showed higher seeds germination and seedling growth rates in the media containing the three composts comparing to control. Toxicity test showed that compost extracts were not toxic. The tested composts might be used as an alternative to peat and the mixtures compost-peat as a growing media for tomato.

**Keywords:** Compost, *Posidonia oceanica*, Chicken Manure, Solid Fraction of Olive Mill Residues, Tomato, Radish, Growth, Germination, Toxicity Test.

**Introduction**

Many authors try to establish the potential value of different compost types as horticultural substrates and nutrient sources (Hartz *et al.*, 1996; Spiers and Fietje, 2000). Several composts from vegetal material were assessed by many authors: Seagrass and seaweed residues were tested with yard waste for horticultural purposes (Orquin *et al.*, 2001), composted green waste was used as a growing medium component (Prasad and Maher, 2001), Roig *et al.* (2001) studied the composting of the solid fraction of olive mill waste water, woodwaste composts were performed as growing media for vegetables under protection by Pudelski (2001). More than one compost from animal material were also studied e.g.: pig manure (Atiyeh *et al.*, 2000), mink manure (Ferguson, 2001),... Production and use of composts not only reduce the volume of wastes but also offer a high potential substrate and reduce usage of peat in the market of growth media (Zoes *et al.* 2001). In this study, we tested the efficiency of three composts, made from Posidonia, chicken manure and solid olive mill wastes, to enhance germination and growth of tomato seedlings in order to replace part of peat in growing media and reduce cost of substrates. Posidonia (*Posidonia oceanica*, Delile) is a Mediterranean marine phanerogam that covers more than two million hectares along the Mediterranean coasts. Leaves accumulated in litter are rejected in enormous quantities on the Tunisian beaches. The chicken manure accumulation is accompanied by serious environmental problems; for instance, the underground water is affected by nitrates and phosphates, air by ammonia emission and soil by accumulated heavy metals and phosphorus

(Walker and Bernal, 2007). The solid fraction of olive mill wastewater is accumulated each year in the Tunisian oil mills and it is very rich in organic matter but its utilisation uses only a small fraction of the available quantities (Fourati *et al.*, 2001). The objective of the present study is to reduce the use of peat as a potting medium by its substitution with composts at different concentrations (0%, 10%, 30% and 50% by volume). Potting media were tested on tomato seedlings and compost extract toxicity was evaluated on tomato and radish seeds.

### Materials and methods

#### Characterization of the original composts

Composts used in this study were produced after 300 days of composting in the Technical Center of Organic Agriculture in Chott Meriem-Sousse (Tunisia). Three composts ( $C_1$ ,  $C_2$  and  $C_3$ ) were tested, with the following combinations: i)  $C_1$ : 50% solid olive mill wastes (SOMW), 20% Posidonia (P), and 30% chicken manure (CM); ii)  $C_2$ : 35% (SOMW), 35% (P), and 30% (CM); iii)  $C_3$ : 20% (SOWM), 50% (P), and 30% (CM). These percentages are proportions in weight. Each compost pile weight was 20 tons. Posidonia was collected from Hergla beach (20 km North from Sousse) and was abundantly washed to eliminate salts from leave surface as suggested by Saïdane *et al.*, (1979). SOMW and CM were collected from an olive mill near Sousse and from a chicken breeding unit in Sousse, respectively. For compost analysis, sampling was made by mixing three subsamples taken from three different points in pile. Each subsample was a mixture of three samples taken from the top to the bottom of the pile at each sampling pile. Samples were placed in polyethylene bags and transferred in the same day to the laboratory (Benito *et al.*, 2005).

Table 1 Physical and chemical characteristics of the three composts  $C_1$ ,  $C_2$  and  $C_3$ .

Parameters	$C_1$	$C_2$	$C_3$
EC	1.64 a ( $\pm 0.31$ )	2.07 b ( $\pm 0.15$ )	1.70 c ( $\pm 0.2$ )
pH	8.23 a ( $\pm 0.03$ )	8.51 b ( $\pm 0.15$ )	8.17 a ( $\pm 0.17$ )
TOC	198.67 a ( $\pm 33.3$ )	207 a ( $\pm 32.97$ )	244.33 a ( $\pm 53$ )
TN	17.56 b ( $\pm 1.1$ )	15.7 ab ( $\pm 1.5$ )	14.53 a ( $\pm 1.33$ )
CaO	66.7 a ( $\pm 11.7$ )	96.76 b ( $\pm 1.36$ )	97.93 b ( $\pm 12.25$ )
MgO	5.04 a ( $\pm 0.87$ )	8.08 ab ( $\pm 0.45$ )	10.05 a ( $\pm 3.05$ )
$K_2O$	2.34 a ( $\pm 0.33$ )	2.68 ab ( $\pm 0.2$ )	4.97 b ( $\pm 1.94$ )
$P_2O_5$	5.09 a ( $\pm 0.54$ )	9.70 b ( $\pm 0.51$ )	13.87 c ( $\pm 3.01$ )
C/N	11.41 a ( $\pm 2.66$ )	13.35 a ( $\pm 3.14$ )	16.69 a ( $\pm 2.35$ )

$C_1$ : 20% *Posidonia oceanica* (Po), 30% Chicken Manure (CM), 50% Solid Olive Mill Wastes (SOMW);  $C_2$ : 35% (Po), 30% (CM), 35% (SOMW);  $C_3$ : 50% (Po), 30% (CM), 20% (SOMW).

EC: Electrical Conductivity ( $mS \cdot cm^{-1}$ ); TOC: Total Organic Matter ( $g \cdot kg^{-1} DW$ ); TN: Total Nitrogen ( $g \cdot kg^{-1} DW$ ); Macronutrient content: CaO: MgO;  $K_2O$  and  $P_2O_5$  ( $g \cdot Kg^{-1}$  of Dry Weight). Values between parentheses are the standard deviations

The samples were analyzed for total organic carbon (TOC) by dry combustion method at 540°C during 4h (Abad *et al.*, 2002) and total N (TN) by Kjeldahl digestion (Bremner and Mulvaney, 1982). Electrical conductivity (EC) and pH were analyzed in a 1:5 (v/v) water

extract. After water extraction (1:5 v/v), K<sub>2</sub>O, CaO and MgO were evaluated by atomic absorption and P<sub>2</sub>O was determined colorimetrically following Murphy and Riley (1962) method. Mean values of three replications on a dry-weight basis (oven dried at 105°C for 24h) were reported. Chemical properties of the investigated composts are summarized in Table 1.

#### Compost extracts

Three extracts (E<sub>1</sub>, E<sub>2</sub> and E<sub>3</sub>) corresponding to C<sub>1</sub>, C<sub>2</sub> and C<sub>3</sub> respectively, were prepared according to the steepage procedure of Weltzein (1992). Each compost was suspended in tap water (1:5 vol/vol) in a loosely covered 10-liter plastic container. The suspension was incubated for 6 days at 15 to 20°C and stirred 5 to 10 min daily. Extracts were filtered through a 2-mm screen to remove large particles, and then preserved in closed flasks at +4°C to be used for the toxicity test.

#### Seedling growth substrates

The seedling growth media consisted of peat as a control substrate (C) and substitutions of peat with 10%, 30% and 50% by volume of composts (S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub>, for each compost concentration respectively). This test gave 9 treatments. Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) was used to determine chemical properties of substrate samples using a Perkin-Elmer Optima 3300 DV ICP-AE Spectrometer. Chemical composition of the growing media is illustrated in table 2.

Table 2 Physical and chemical characteristics of the assessed substrates (peat substituted with different concentrations of three composts C<sub>1</sub>, C<sub>2</sub> and C<sub>3</sub>).

Parameters	Control B	10%	S1 30%	50%	10%	S2 30%	50%	10%	S3 30%	50%
<i>pH</i>	6 a (±0.09)	6.91 d (±0.04)	6.86 c (±0.07)	6.74 cd (±0.06)	6.88 cd (±0.06)	6.84 cd (±0.04)	6.77 cd (±0.07)	6.83 cd (±0.09)	6.78 cd (±0.01)	6.53 b (±0.01)
<i>EC</i> (mS/m)	30 a (±0.21)	132.0 b (±0.7)	158.3 d (±0.52)	170.0 g (±0.82)	163.5 e (±0.36)	187.0 h (±0.43)	201.2 i (±0.7)	132.8 b (±0.78)	148.0 c (±0.79)	165.6 f (±0.7)
<i>Na</i> (mg/g)	0.38 a (±0.004)	0.21 e (±0.004)	0.18 b (±0.02)	0.48 g (±0.009)	0.19 d (±0.002)	0.18 c (±0.001)	0.6 i (±0.006)	0.6 i (±0.006)	0.25 f (±0.004)	0.57 h (±0.004)
<i>Mg</i> (mg/g)	0.16 a (±0.002)	1.26 c (±0.019)	1.25 c (±0.004)	1.13 b (±0.002)	1.41 f (±0.018)	1.45 g (±0.009)	1.62 h (±0.012)	1.31 d (±0.011)	1.65 i (±0.018)	1.38 e (±0.008)
<i>K</i> (mg/g)	0.43 a (±0.005)	1.75 ef (±0.028)	1.60 d (±0.011)	1.18 b (±0.021)	1.60 de (±0.017)	1.40 c (±0.002)	1.68 de (±0.016)	1.44 c (±0.012)	1.77 f (±0.024)	1.54 de (±0.011)
<i>P</i> (mg/g)	0.12 e (±0)	0.16 a (±0.001)	0.17 b (±0)	0.24 c (±0.004)	0.43 g (±0.008)	0.64 h (±0.001)	0.36 f (±0.005)	0.25 d (±0.002)	0.65 h (±0.006)	0.34 e (±0.001)

S<sub>1</sub>: mixture peat and C<sub>1</sub>, S<sub>2</sub>: mixture peat and C<sub>2</sub> and S<sub>3</sub>: mixture peat and C<sub>3</sub>;

A: Means within the same line followed by the same letter are not significantly different at *P* = 0.05.

B: Control represent 100% of peat.

Values between parentheses are the standard deviations

### Plant growth experiment

Twenty tomato seeds (var. Rio Grande) were sown in alveolus plates containing peat substituted with 0% (control), 10%, 30% or 50% by volume of composts (substrates S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> respectively). Plates were put in a glasshouse at 23°C and watered daily. The number of seedlings successfully emerging in each alveolus plate was weekly recorded during five weeks. At the end of the fifth week, five plants from each alveolus plate were randomly chosen, separated into shoot and root portions and dried at 60°C for 3 days to determine their dry weight. Mean values of three replicates were recorded.

### Toxicity test

Before suggesting the use of such composts for field cultivation, their toxicity was tested on Tomato (*Lycopersicum esculentum*, L. var. Rio Grande) and Radish (*Raphanus sativus* L. var. National) seeds. These two species seeds were disinfected in absolute ethanol during 5 min. They are then rinsed with sterile distilled water to eliminate traces of pesticides that might be used in seed treatments (Benhamou *et al.*, 1997), and placed in Petri dishes (Ø 8 cm) lined with filter paper containing 6 ml of the three obtained aqueous extracts (E<sub>1</sub>, E<sub>2</sub> and E<sub>3</sub>). Petri dishes were incubated in a growing chamber at 26°C. Seeds were maintained soaked with the corresponding composts extracts and distilled water was used as control. During 7 days, germinated seeds were counted and at the seventh day, root and shoot lengths were measured and mean values were recorded. Three Petri dishes were replicated for each treatment and in each Petri dish, five seeds were used.

### Statistical analysis

Mean values of all the studied parameters were compared by variance analysis (ANOVA), using the SPSS 16.0 (SPSS Inc., 2007), and differences between the means were determined using the Duncan test. Means were separated on the basis of least significant difference at 5% probability level.

## Results and Discussion

### Compost effect on physical and chemical properties of potting media

Upon the substitution of 10%, 30% and 50% composts into peat, electrical conductivities of potting media increased with the increasing concentrations of the three assessed composts (Table 2). While pH values of the potting mixtures remained almost unchanged with increasing substitutions of the tested composts. And recorded values of Mg, K, Na and P were not correlated with the compost concentrations (Table 2).

### Seedling emergence

Figure 1 shows an evolution of seedling emergence percentage during five weeks which was obvious from the third week in all substrates. At the third week, in control treatment (C), we registered significantly lower percentage emergence (66.7%). Whereas, the mean percentage of seedling emergence obtained in the three assessed treatments was 69% and 57% at 10% and 50% respectively and at 30% compost concentration, the highest rate of seedling emergence was in S<sub>1</sub> (71%). By the end of the experiment (fifth week), emergence was far more complete in all treatments with more than 80% of emerged seedlings. However, significant differences between all treatments and control were noted.

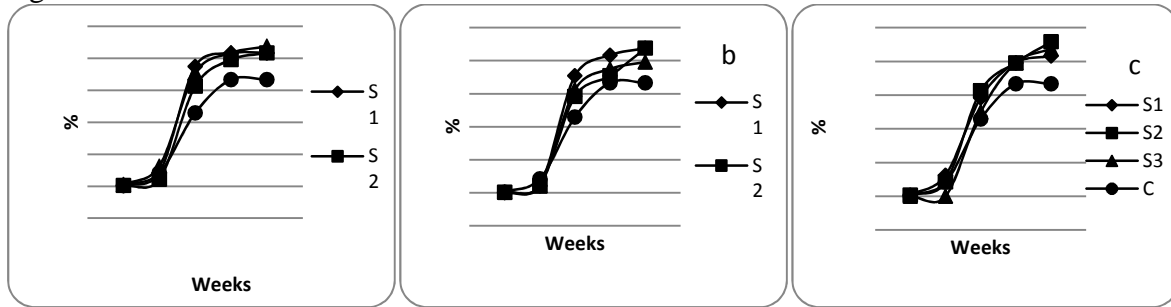


Figure 1 Seedling emergence percentages in the four substrates (S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> and control C) at the three composts concentrations (a: 10%; b: 30% and c: 50%). S<sub>1</sub>: mixture peat and C<sub>1</sub>, S<sub>2</sub>: mixture peat and C<sub>2</sub> and S<sub>3</sub>: mixture peat and C<sub>3</sub>. Vertical bars show the average standard deviations

### Seedling growth

Growth rates were comparable for all treatments and assessed concentrations of composts (Fig. 2). At the three assessed concentrations, there were significant differences between the three composts applied and the control. Statistical analysis showed that the difference of seedling length was significant between mixed substrates and peat. This difference appeared after two weeks of growth. Seedlings growth was also estimated by root and shoot dry weights (Table 3). Root and shoot dry weights measured at the assessed concentrations of the three composts were significantly higher than control and the highest values were recorded at 50% of composts for root dry weight and at 10% for shoot dry weight.

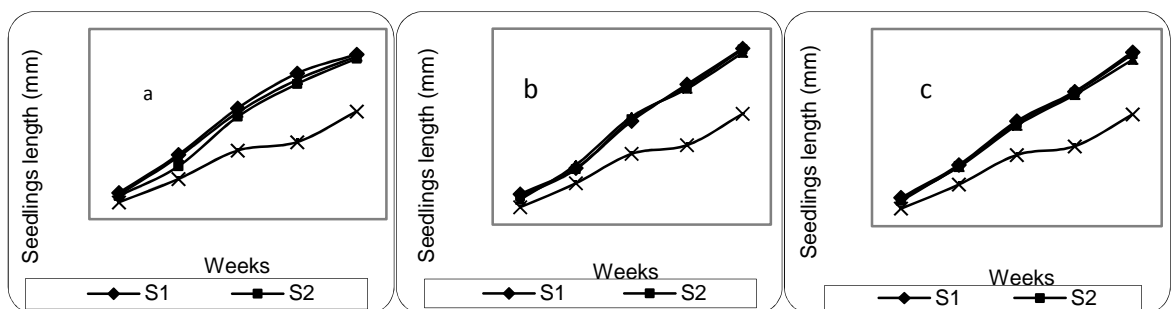


Figure 2 Growth of tomato seedlings in the four substrates (S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> and control C) at the three composts concentrations (a: 10%; b: 30% and c: 50%). S<sub>1</sub>: mixture peat and C<sub>1</sub>, S<sub>2</sub>: mixture peat and C<sub>2</sub> and S<sub>3</sub>: mixture peat and C<sub>3</sub>. Vertical bars show the average standard deviations

Table 3: Final dry weight of tomato roots and shoots five weeks after germination in the four substrates (S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub> and control C) at the three concentrations of composts (10%, 30% and 50%)

Substrates Concentrations	S1			S2			S3			
	Control B	10%	30%	50%	10%	30%	50%	10%	30%	50%
Root DW (g)	0.41a (±0.08)	0.63 bc (±0.04)	0.63 bc (±0.07)	0.73 cd (±0.1)	0.59 b (±0.04)	0.57 b (±0.06)	0.86 e (±0.03)	0.61 bc (±0.04)	0.55 b (±0.04)	0.77 de (±0.09)
Shoot DW (g)	3.1 a (±0.06)	6.65 g (±0.12)	4.55 c (±0.1)	5.65 e (±0.17)	5.55 e (±0.16)	4.65 c (±0.09)	5.15 d (±0.11)	6.05 f (±0.18)	3.65 b (±0.11)	5.45 e (±0.08)

A: Means within the same line followed by the same letter are not significantly different at  $P = 0.05$ .

B: Control represent 100% of peat

### Toxicity test

The high rates of tomato and radish seed germination in all treatments suggests that compost extracts are not phytotoxic. Mean values of seed germination percentages in all treatments were at least 98% for radish seeds and 100% for tomato seeds and were not significantly different from germination in control (100%) at the seventh day (Fig. 3a and 3b). Seed germination was not delayed by compost extracts for tomato and radish. Since the first day, tomato seed germination was accelerated in C<sub>2</sub> and C<sub>3</sub> extracts with respective germination rates of 80% and 90% (fig. 3b). Concerning radish seeds, there was no significant difference between the germination speed in all treatments (Fig. 3a). Compost extracts did not affect shoot length of radish (Fig. 4a) but they increased tomato shoot length in all treatments (ranged between 32.7 mm and 36.9 mm). Notice that the shoot length of control did not exceed 19.3 mm, and root length was not affected by compost extracts in almost all treatments for the two tested species (fig. 4b).

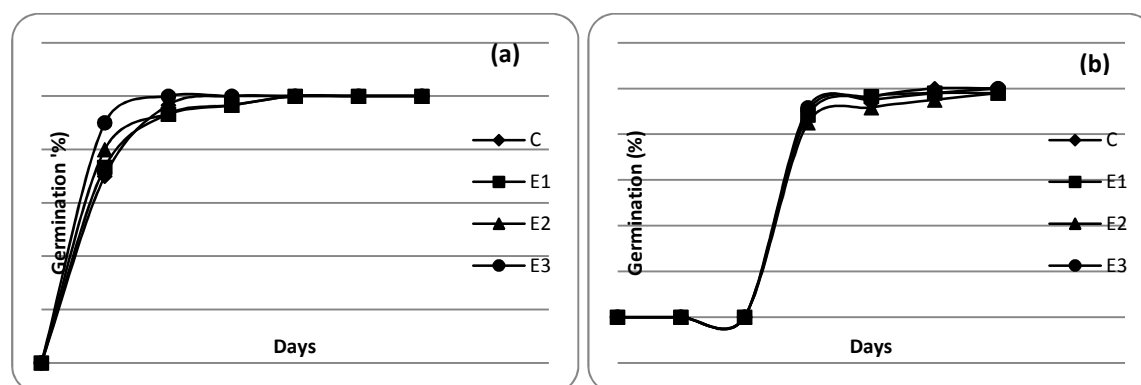


Figure 3 Germination kinetic of Radish (a) and Tomato (b) seeds in four compost extracts (E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>) and control (C). E<sub>1</sub>: C<sub>1</sub> extract, E<sub>2</sub>: C<sub>2</sub> extract and E<sub>3</sub>: C<sub>3</sub> extract

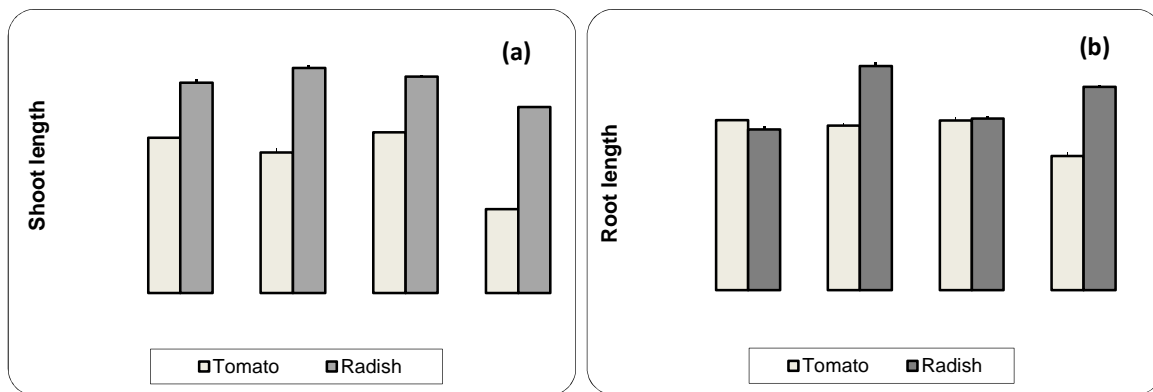


Figure 4 Radish and Tomato shoot (a) and root (b) lengths (mm) in presence of compost extracts E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub> and control C. E<sub>1</sub>: C<sub>1</sub> extract, E<sub>2</sub>: C<sub>2</sub> extract and E<sub>3</sub>: C<sub>3</sub> extract

There is a positive correlation between substrates electrical conductivity (EC) values and the compost concentrations, which corresponded with results reported by Klock (1997). EC values ranged between 132 and 201 mS/m, nevertheless substrates tested on tomato seedlings were not phytotoxic. This differs from the results of Gejdos (1997) who reported that salinity could be an adverse factor when it exceeded 100-300 mS/m. The relatively high pH values of the original composts were far from the optimum pH for growing media (5.2 - 6.3) given by Bunt (1988). However, these values do not seem to be hazardous for tomato plants even though their growing rate is better in acid media (Brady and Weil, 1999). The pH values of the potting mixtures remain almost unchanged with increasing substitution of composts. This result does not confirm the findings of Tyler *et al.* (1993) who reported increases in the substrate pH with increasing concentrations of composted turkey litter added to the potting medium. According to Levy and Taylor (2003), C/N ratios recorded in this study, which were between 11.3 and 16.8, might be considered suitable for vegetable crops. Abad *et al.* (2001) mentioned that above 80% of organic matter should be adequate for potting media. Thus, the growing media used in this essay might be considered suitable. The three composts used in this work produced conspicuous differences in growth of young tomatoes from 10% by volume, compared with peat. This improvement of growth might be attributed to richness of the composts in nitrogen and other nutrients. The increasing of tomato seedling biomass was studied by Subler *et al.* (1998) who reported that the incorporation of 10% by volume of pig manure vermicompost increased significantly the total biomass of tomato after three weeks of growth. Bugbee and Frink (1989) also mentioned that when a container medium was replaced with 10%, 20%, 30%, 40% or 50% (by volume) of sewage sludge compost, shoot dry weights and plant growth of marigold plants improved significantly, especially in 30% compost containing mixture. The phytotoxicity tests show that all compost extracts are suitable for germination and growth of Tomato and Radish, in spite of the well known sensitivity of radish to plant extracts (Tsuzuki *et al.*, 1995). We conclude that composting of *Posidonia* mixed with chicken manure and olive mill residues shows some interesting outcomes and the final product may be a substitute for peat as the organic component of potting media.

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## EFFECT OF ACID SOILS FERTILIZATION ON MORFOLOGICAL AND PRODUCTIVE CHARACTERISTICS OF TRITICALE

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### Abstract

This paper presents the results of some morphological and productive characteristics in two cultivars of triticale depending of fertilization systems of acid soils.

The experiment is included three different fertilizer rates and two triticale cultivars (KG-20 and Tango). The I variant of fertilization included NPK 80:80:60 combinations, II variant is a combination of nutrients and had 80:100:60, and the III variant with NPK fertilizer 80:80:60 had added 4 t ha<sup>-1</sup> of lime fertilizer “Njival Ca” and 20 t ha<sup>-1</sup> of manure. With the use of mineral fertilizers with a higher dose of phosphorus, as well as a combination of mineral fertilizers with lime and organic fertilizers, there was a significant increase in all morphological and productive characteristics of triticale. The highest yield of both cultivars was achieved with the one that had a combination of mineral, lime and organic fertilizers. The yield of cultivar Tango in that variant was 7.41 t ha<sup>-1</sup>, and cultivar KG-20 6.68 t ha<sup>-1</sup>. This yield was significantly higher than the yield achieved in the variant I, where was applied mineral fertilizer 80:80:60. Statistically, there were no significant differences in grain yield between variants I and II, and between II and III. Also, differences were apparent between cultivars. Plant height, spike`s length, absolute grain mass, hectoliter grain mass and grain yield were higher in Tango cultivar in relation to the cultivar of KG-20.

**Key words:** triticale, fertilization, absolute mass, hectoliter mass and yield.

### Introduction

Triticale is a cross between wheat and rye with the idea to combine the high level of proteins in wheat with high yield and quality of rye proteins. (Radecki & Miller, 1990),

According to many researchers (Borojevi , 1981; Cvetkov, 1982; oki , 1988) triticale has a high genetic potential for high yield and good nutritional value and is considered to be a promising plant species.

To achieve high and stable yields it is necessary to have favorable agro-climatic conditions of the area, assortment and fertilization.

In our country, acidic soil is a serious problem in crop production. These soils are poor in water-air and physical-mechanical characteristics, and on these soils crop production is unstable. According to Aniola and Madeja (1996) the highest tolerance to acid soils exhibit rye, triticale and wheat, while barley is the most sensitive. Numerous studies here and abroad show that appropriate application of lime fertilizers in combination with organic and mineral is the most effective way to eliminate unfavorable production characteristics of acid soils and affect in yield increase. (Jovanovi et al., 2006; Kova evi et al., 2006; Jeli et al., 2006).

When choosing the type and quantity of fertilizer it is necessary to take into account the state of soil fertility. In order to achieve the same yield it is necessary to put less fertilizer on fertile soils than on poor soils. The aim of our study was to determine some morphological and

productive characteristics of triticale on acid soil, depending on the system of fertilization and variety.

### Materials and methods

Tests were conducted at the Center for Agricultural and Technological Research in Zaje ar municipality during 2008/09 and 2009/10. The experiment is situated like block design in three replications and it included two cultivars of triticale (KG-20 i Tango) and three variants of fertilization. The I variant of fertilization included NPK feed 80:80:60 combinations, II variant is a combination of feed and had 80:100:60 combination, and the III variant with NPK fertilizer 80:80:60 had added 4 t ha<sup>-1</sup> of lime fertilizer “Njival Ca” and 20 t ha<sup>-1</sup> of manure. Primary treatment was performed in a classical way (to 25 cm depth) immediately after corn harvest and corn removal. Sowing was in October. Triticale harvest was performed in the stage of full maturity, when plant height, spike`s length, number of grains in spike, absolute grain mass, hectoliter grain mass and grain yield were determined. The yield was adjusted to 14% moisture. Results are presented as the average two-year and were analyzed statistically using analysis of variance.

#### Soil and climatic conditions

The following table shows an overview of chemical properties of soil.

Table 1. Chemical characteristics of soil

Zaje ar municipality- vertisol					
Depth (cm)	pH		N (%)	Easyaccessible ( mg/100 g soil)	
	H <sub>2</sub> O	nKCl		P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
0-20	5.23	4.84	0.12	16.68	29.53
20 - 40	5.54	5.15	0.11	12.34	27.22

Soil in Zaje ar municipality is non-calcareous vertisol type and is characterized by high acidity (pH u KCl-u 4,84 -5.15). The nitrogen content in the profile to 20 cm is 0.12% and decreases with depth. The content of available phosphorus in profile up to 20 cm is 16,68 mg /100 g, and in the deeper layers 12.34 mg. This soil is very rich in potassium (29,53 mg/ 100 g in the upper layers). It belongs to the category of minute soils, which means that the optimal deadline for processing is very short. It has to be improved, in order to have good yield.

Table. 2. Meteorological conditions during the experiment (2008-2010)

Months	Average monthly temperature of air (°C)		Monthly sum of precipitation (mm)	
	2008/09	2009/10	2008/09	2009/10
X	11.9	11.0	28.2	106.2
XI	6.1	7.3	29.9	106.3
XII	1.8	1.1	98.1	123.1
I	-1.3	-2.2	67.3	54.1

II	1.3	0.4	91.1	108.0
III	6.0	6.0	58.3	64.3
IV	12.1	11.9	15.4	73.5
V	17.8	16.6	18.0	58.9
VI	20.6	20.8	76.4	95.1
Average Sum	<b>8.4</b>	<b>8.1</b>	<b>482.7</b>	<b>789.5</b>

Average monthly temperatures, in both years, were similar. Temperatures were within optimal limits and did not have the impact on yield. Higher rainfall was in 2009/10, which resulted with better yield compared with the previous year. During October, November and December 2009/10 rainfall was 335.6 mm resulting higher winter moisture. In the same year, higher rainfall in Junu did not have good impact on triticale, when the plants were at the stage of grain filling and ripening. Rainfall distribution during the growing season, was different every year.

### Research results and discussion

#### Morphological characteristics

Morphological characteristics were mainly varietal characteristics, although depend on the conditions of production and agrotechnics. Table 3. presents some morphological characteristics of triticale depending on fertilizing system.

Table. 3. Some morphological characteristics of triticale depending on fertilizing system.

Fertilization variants	Morphological characteristics					
	<i>Plant's height (cm)</i>		<i>Spike's length (cm)</i>		<i>Number of grains in spike</i>	
	KG-20	Tango	KG-20	Tango	KG-20	Tango
0. Control	62.0	75.2	7.0	9.0	31	34
I. NP <sub>1</sub> K	92.0	102.4	9.2	11.9	36	40
II. NP <sub>2</sub> K	91.3	102.8	9.2	12.7	36	42
III. NP <sub>1</sub> K+CaCO <sub>3</sub> +manure	92.0	102.5	9.9	13.0	38	43
Average	<b>84.3</b>	<b>95.7</b>	<b>8.8</b>	<b>11.7</b>	<b>35.2</b>	<b>39.7</b>
LSD- test						
5 %	4.32	4.84	0.78	0.82	2.17	2.51
1 %	5.13	5.56	0.92	0.95	2.49	2.73

Plant height is varietal characteristic and largely depends on the conditions of production. It is very important for plant lodging. Lower stem plants have greater resistance to bad weather conditions. Average triticale height was 84.3 cm in cultivar KG-20 up to 95.7 cm in cultivar Tango. In both cultivars, plant height in all variants of fertilization, was significantly higher compared to the control variant, while between the variants of fertilization there were no statistically significant differences.

Spike's length and number of grains in spike are characteristics with significant affect on yield. Average spike's length was from 8.8 cm in cultivar KG-20 up to 11.7 cm in Tango cultivar. In all variants, in both cultivars, plants had significantly higher spike's length than

plants in control variant. Tango cultivar spike's length in variant III was significantly higher than in the variant I. Average number of grains in spike was 35.2 in cultivar KG-20 up to 39.7 in Tango cultivar. Both cultivars in variants with fertilization had significantly greater number of grains in spike, than in control variant, while Tango cultivar had greater number of grains in spike in variant III than in variant I. It was concluded that there were no statistically significant differences between variants I and II and between variants II and III in all examined characteristics. Tango cultivar in all variants of fertilization had higher values than cultivar KG-20 in examined characteristics. Wiegand and Cuellar (1981) assert that number of spikes and number of grains in spike are very important for yield which needs good weather conditions in different growth stages.

#### Productive characteristics

Productive characteristics directly affect the yield. Especially important is the advantage of agro-climatic factors. Table 4. provides an overview of some productive characteristics depending on fertilization system.

Table. 4. Some productive characteristics of triticale depending on fertilization system

Fertilization variants	Productive characteristics					
	Absolute mass (g)		Hectoliter mass (g)		Grain yield (t ha <sup>-1</sup> )	
	KG-20	Tango	KG-20	Tango	KG-20	Tango
0. Control	42.1	43.2	68.8	70.3	2.25	3.00
I. NP <sub>1</sub> K	41.0	43.0	70.0	74.3	5.95	6.85
II. NP <sub>2</sub> K	42.3	43.2	70.2	74.6	6.10	7.05
III. NP <sub>1</sub> K+CaCO <sub>3</sub> +manure	43.0	44.0	70.4	74.0	6.68	7.41
Average	<b>43.1</b>	<b>43.3</b>	<b>69.8</b>	<b>73.3</b>	<b>5.24</b>	<b>6.07</b>
LSD- test						
5 %	3.62	3.43	4.13	4.28	0.59	0.54
1 %	3.72	3.61	4.67	4.91	0.68	0.68

Absolute grain mass is grain size indicator and mostly depends of plant density and climate factors. Triticale have large grain (40-65 g) larger than wheat and rye (Pržulj et al.,1989).

In our researches, between fertilization variants and between cultivars there were no statistically significant differences in absolute grain mass. It is important to emphasize that absolute grain mass in control variant was approximately equal to the one in fertilized variants. This is justified with the fact that in the control variant plants were thinner and spikes had less grains which were replenished and massy. Wiegand and Cuellar (1981) emphasize that yield primarily depends on grain mass.

Average hectoliter grain mass was 69.8 kg in cultivar KG-20 up to 73.3 kg in Tango cultivar. Differences in hectoliter mass were bigger between the cultivars than in between fertilization variants, especially in cultivar KG-20. Statistically significant difference in hectoliter grain mass, in Tango cultivar, was between variants I and II comparing to control variant which had significantly lower grain mass.

Grain yield is very important category for all manufacturers. Grain yield is influenced by a number of factors and the most important are cultivar, agrotechnics and climate. If we compare yield with fertilizers and without fertilizers (control), the highest yield in both cultivars was in variant with the fertilizers. Average yield in cultivar KG-20 was 5.24 t ha<sup>-1</sup> and in Tango cultivar 6.07 t ha<sup>-1</sup>. The lowest yield was in control variant and was

significantly lower than in variants with fertilizers. The highest grain yield in both cultivars ( $6.68 \text{ t ha}^{-1}$  i  $7.41 \text{ t ha}^{-1}$ ) was in variant III, with combination of mineral, lime and organic fertilizers. Yield was significantly higher than in variant I. The difference in the highest yield (variant III) between Tango and KG-20 cultivar was  $730 \text{ kg ha}^{-1}$  in favour of Tango cultivar. There were no significant differences in grain yield in both cultivars between variants I and II, and between II and III. Considering that there were no statistically significant differences in grain yield between variants II and III, variant (II) with increased phosphorus content is recommended as the most rational considering the price of lime and organic fertilizers. Other authors (Jeli et al., 1998; Jovanovi et al., 2006; Kova evi et al., 2006) also had positive effect, with increased doses of phosphorus fertilizers, on wheat yield. Numerous previous studies and our results have shown that on acid soils usage of NPK, lime fertilizer and manure have positive effect on grain yield (Ognjanovi et al., 1994; Jeli et al., 1995; Jeli et al., 2004)

### Conclusion

The study of the influence of fertilization systems and the cultivars on morphological and productive characteristics of triticale showed:

Fertilization systems and cultivars had significant effect on morphological and some productive characteristics of triticale.

Plant height, spike's length and number of grains in spike, in both cultivars, were significantly higher in variants with fertilizers.

Determined values of morphological characteristics were higher in Tango cultivar than in cultivar KG-20.

There were no significant differences in both cultivars, in absolute grain mass between control and fertilization.

Hectoliter mass, in cultivar KG-20, showed no significant differences, both between control and fertilization, nor between fertilization variants.

Tango cultivar in a variant with higher dosage of phosphorus had significantly higher hectoliter grain mass compared to the control variant.

Grain yield, in both cultivars, was much higher in variants with fertilizer than in control variant.

The highest yield, in both cultivars, was in variant with combination of mineral, lime and organic fertilizer.

There were no statistically significant differences in grain yield between variants III and II, Variant (II) with increased phosphorus content is recommended.

According to all parameters, Tango cultivar was better than KG-20. It is recommended for growing in this area.

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## GRAIN YIELD AND YIELD COMPONENTS OF TRITICALE ON AN ACID SOIL DEPENDING ON MINERAL FERTILISATION AND LIMING

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### Summary

A field experiment with triticale cvs. 'Tango', 'KG-20' and 'Odyssey' was established on a pseudogley (gleysol) in 2010/11 and 2011/12 to evaluate the effect of different rates of mineral fertilisers and lime on grain yield and yield components. Mineral fertilisation and liming led to a significant increase in grain yield components, notably grain number and grain weight per spike, thus resulting in increased grain yields in fertilised treatments. Liming gave a higher grain yield as compared to the increased P rate, mostly due to P immobilisation in the highly acidic environment. Growing triticale on very acid soils should involve liming, if considered economically feasible, to increase soil pH above the suboptimal level (pH 5.0) for the realisation of its yield potential.

**Keywords:** triticale, grain yield, mineral fertilisation, liming, acid soils

### Introduction

Triticale (*x Triticosecale*) is a small grain crop produced by crossing wheat (*Triticum sp*) and rye (*Secale cereale*) to combine the high yield potential and good grain quality of wheat with the resistance/tolerance to biotic and abiotic stresses of rye. The nutritional advantages of triticale over maize make triticale an increasingly preferred feed for all types of livestock (Čekić et al., 2011). Moreover, the potential use of triticale as a complete or partial substitute for commercially important cereal grains (wheat and maize) in fish (carp) feeds has been confirmed (Marković et al., 2012).

Through the improvement of the most important economic traits viz. grain yield, nutritional quality, early maturity and grain fill, triticale has become an increasingly attractive option, particularly for regions with cold climates and for low-fertility, degraded and low-pH soils.

It has been estimated that 30-40% of the world's arable land is acidic, with a pH below 5.5 (von Uexkull and Mutert, 1995). There are many chemical limitations and interactions among chemical compounds in acid soils that constrain plant growth. Acid soils are deficient in Ca, Mg and Mo, low in available P, and have increased levels of toxic H, Al and Mn ions, with Al toxicity being the major constraint to plant growth.

Plant species and cultivars within species vary widely in their resistance to Al toxicity. A relatively wide range of Al resistance has been observed in wheat (de Sousa, 1998) and rye (Aniol et al., 1980). Therefore, triticale is also expected to show markedly different tolerance to soil acidity. Triticale has moderate soil requirements and good tolerance of low pH (about 5.0), and a large number of its strains show better adaptability to acidity compared to wheat cultivars (Oettler et al., 2000; Madic et al., 2013).

The objective of this study was to evaluate the effect of mineral fertilisation and liming on the grain yield and yield components of three triticale cultivars grown on an acid soil.



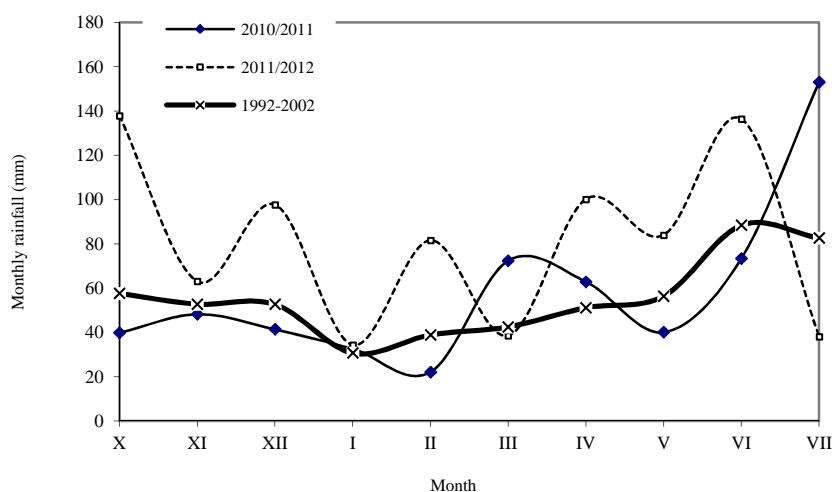
## Material and methods

The research was conducted at the experimental field of the Secondary School of Agriculture and Chemistry, Kraljevo, in 2010/2011 and 2011/2012. The experiment was established on an acidic pseudogley (gleysol),  $\text{pH}_{\text{H}_2\text{O}}$  4.5, having a humus content of 2.2%. Triticale cultivars 'Tango' (Agricultural and Technological Research Centre, Zaje ar), 'KG-20' and 'Odyssey' (Small Grains Research Centre, Kragujevac) and different fertilisation treatments were used (Table 1).

Table 1. Fertiliser rates used in the experiment ( $\text{kg ha}^{-1}$ )

	Fertilisation ( $\text{P}_2\text{O}_5 \text{ kg ha}^{-1}$ ) : for the fertilised treatments = + 120 N + 53 $\text{K}_2\text{O}$			
	$\text{P}_0$ (unfertilised)	$\text{P}_1$	$\text{P}_2$	$\text{P}_1\text{Ca}$
$\text{P}_2\text{O}_5$ ( $\text{kg ha}^{-1}$ )	0	80	180	
$\text{CaCO}_3$ ( $\text{t ha}^{-1}$ )	0	0	0	5

The experiment was laid out as a randomised block design in three replications, with a plot size of  $5\text{m}^2$ . Sowing was conducted in mid-October at a row spacing of 12.5 cm and a within-row spacing of 3 cm. Fertilisation involved the use of complex NPK fertilisers (8:24:16), superphosphate (17%  $\text{P}_2\text{O}_5$ ) and ammonium nitrate (34.4% N) as a nitrogen fertiliser for top dressing. Prior to seedbed preparation, the whole amount of phosphorus and potassium fertilisers and a third of the nitrogen fertiliser were manually broadcast. Treatment  $\text{P}_1\text{Ca}$  included the broadcast application of "Njival Ca" lime fertiliser (98.5%  $\text{CaCO}_3$ , 1%  $\text{MgCO}_3$ ). The rest of the nitrogen was used as a single dressing in early spring. At full maturity, a sample consisting of 30 plants was collected from each plot for analysis of plant height, spike length, grain number per spike and grain weight per spike. After harvest, grain yield was weighed and calculated in  $\text{kg ha}^{-1}$ . The results were subjected to analysis of variance, using SPSS software (1995). The significance of differences between means was assessed by the LSD test.



Graph 1. Monthly rainfall during the experimental growing season, and the long-term average (1992-2002) for Kraljevo

The total rainfall in both growing seasons was comparable to the long-term average, with a rather uneven distribution across months (Graph 1). At sowing, emergence and other critical stages for plant growth, total rainfall was sufficient to ensure intensive plant growth and development.

### Results and discussion

The analysis of variance for all traits shows significant differences among cultivars (except for grain yield in the second year) and fertilisation treatments, as well as variations across cultivars in their response to fertilisation (cultivar/fertilisation interaction) (Table 2).

The differences among cultivars were mostly consistent across years; plant height, spike length and grain number/weight per spike were significantly lower in cv. 'KG-20' than in the other cultivars tested (Table 2).

Plant height in all cultivars was the lowest in the unfertilised treatment (P1), whereas spike length and grain number/weight per spike were significantly higher in all fertilised treatments than in treatment P0. In both years, spike length was significantly lower in treatment P0, compared to the other treatments. In the first year, grain number per spike was the lowest in treatment P0, significantly higher in treatment P1, and highest in treatments P2 and P1Ca; in the second year, significantly higher values were found in all treatments compared to treatment I. In both years, grain weight per spike was the lowest in treatment P0, significantly higher in treatment P1, and the highest in treatments P2 and P1Ca.

Regardless of fertilisation, differences in grain yield were observed only between 'Tango' and 'Odyssey' in the first year. Differences in grain yield in both years, regardless of cultivar, were significant across all treatments; the lowest yield was produced in treatment I, and the highest in treatment P1Ca.

In both years, the use of NP1K (treatment P1) led to an almost twofold increase in grain yield in all cultivars compared to the unfertilised treatment.

Increasing rates of P (treatments P1 and P2) resulted in 18.2% and 17.3% increases in grain yield in the first and second years, respectively. Liming (treatments P1 and P1Ca) increased grain yields by 36.6% in the first year and 32.1% in the second year. The marked response of the test cultivars to lime, compared to increased P application rates, is associated with P immobilisation in the very acidic environment (Edwards et al.1991). The cultivars showed consistent responses to increased P rates and liming in both years. Grain yield of triticale significantly increased with increasing combined N+P fertiliser rates up to 200 kg N+ 40 kg P ha<sup>-1</sup>, whereas the positive response to further increases in NP rates progressively diminished, leading to a negative effect of overfertilisation (Moinuddin and Afridi, 2008). Harmoney and Thompson (2005) also reported a favourable response of triticale (grown for green forage) to increasing N and P fertilisation up to a certain rate, whereas StošoviC et al (2010) suggested positive effects of increased N rates on grain yield, with the rate of 80 kg N ha<sup>-1</sup> being the most cost-effective for large-scale production.

The average grain number per spike was higher in fertilised treatments than in treatment I by 40.6% in the first year and 41.3% in the second year, whereas grain weight per spike increased by 51.5% and 78.8%, respectively. The marked increase in grain weight per spike in fertilised treatments, compared to grain number per spike, was attributed to favourable conditions in the grain filling period, primarily sufficient soil moisture, particularly in the second year. Different environments can modify the expression of yield components, thus making changes in both grain yield and the relative contribution of yield components to grain yield (Wallace and Zobel, 1994). The results obtained confirm the finding of Giunta et al. (1999) that grain fill duration, moisture and assimilate supply during the period are the key determinants of grain weight.

Table 2. Results of investigations

Impacts of cultivar (factor A: A1 = Tango, A2 = KG-20, A3 = Odyssey) and fertilisation (factor B: B1 = unfertilised; B2 = P1, B3 = P2; B4 = P1Ca: Table 1) on plant height (PH), spike length (SL), grain number per spike (GNS), grain weight per spike (GWS) and grain yield (GY) of triticale

Factor		PH	SL	GNS	GWS	GY	PH	SL	GNS	GWS	GY
A	B	cm	cm		g	kg ha <sup>-1</sup>	cm	cm		g	kg ha <sup>-1</sup>
2010/2011 growing season						2011/2012 growing season					
A1		110.8a	10.6a	45.9a	1.82a	4378	113.9a	10.7a	44.9a	1.85a	4378
A2		99.6b	8.4b	36.0c	1.37b	4046	101.9b	7.7b	34.2c	1.15c	4046
A3		107.4a	10.9a	39.4b	1.43b	4089	116.2a	11.2a	37.2b	1.59b	4089
	P0	90.4c	7.5c	31.0c	1.11c	2101d	99.2b	8.0 bc	29.3b	0.96c	2101d
	P1	102.5b	10.1b	40.2b	1.50b	4173c	110.9a	10.6 a	43.0a	1.61b	4173c
	P2	114.2a	10.7ab	43.9a	1.73a	4895b	117.5a	10.7a	41.4a	1.67b	4895b
	P1Ca	116.8a	11.6a	46.7a	1.82a	5514a	115.1a	10.3a	41.4a	1.87a	5514a
A1	P0	89.3	7.1c	32.7	32.7	2081	95.7d	8.0bc	1.17d	1.17d	2081
	P1	108.4	11.1ab	49.2	49.2	4175	117.2a	11.8a	1.94ab	1.94ab	4175
	P2	122.0	12.0a	51.3	51.3	4935	125.0a	12.2a	2.04ab	2.04ab	4935
	P1Ca	123.6	12.4a	50.5	50.5	6301	117.8ab	10.9a	2.24a	2.24a	6301
A2	P0	85.8	7.2c	29.5	29.5	1999	99.1cd	6.9c	0.86e	0.86e	1999
	P1	96.0	8.2c	35.3	35.3	3983	94.1d	7.9bc	1.17d	1.17d	3983
	P2	106.6	8.1c	36.9	36.9	4797	105.3cd	7.5bc	1.04de	1.04de	4797
	P1Ca	110.0	10.0b	42.4	42.4	5404	109.1bc	8.4b	1.54c	1.54c	5404
A3	P0	95.9	8.3c	30.9	30.9	2224	102.9cd	8.6b	0.86e	0.86e	2224
	P1	103.1	11.0ab	36.1	36.1	4360	121.5a	12.2a	1.72bc	1.72bc	4360
	P2	113.9	12.1a	43.6	43.6	4935	122.1a	12.3a	1.93b	1.93b	4935
	P1Ca	116.8	12.4a	47.1	47.1	4838	118.3a	11.8a	1.84bc	1.84bc	4838
ANOVA											
	A	**	**	**	**	ns	**	**	**	**	ns
	B	**	**	**	**	**	**	**	**	**	**
	AB	ns	*	ns	*	ns	*	*	**	*	ns

Mean values designated with the same lowercase letter are not significantly different at the 95% level according to the LSD test

\*\* F-test significant at 0.01; \* F-test significant at the 0.05 level; ns non-significant

### Conclusion

Mineral fertilisation and liming led to a significant increase in spike length, grain number per spike and grain weight per spike. These yield components are major determinants of grain yield; therefore, yield was significantly higher in fertilised treatments. Soil liming gave a higher grain yield as compared to increasing P rates, due to the greater availability of macro- and micronutrients at increased soil pH or due to P immobilisation in the highly acidic environment. Growing triticale on very acidic soils should involve liming, if considered economically feasible, to increase soil pH above the suboptimal level (pH 5.0) for the realisation of its grain yield potential.

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**THE EVALUATION OF MORPHOLOGICAL, BIOLOGICAL AND PRODUCTIVE  
OF THE MAIZE HYBRIDS**

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**Abstract**

The maize is the most prevalent crop in the world. It is considered a green mine, because for a short time, 100-135 days, it gives a dry mass production (grain and green mass), that can't be completed by any other plant. It has high and diverse values of use. Studies in maize plants are numerous, and they continue for different aspects: genetics, breeding, improving of technological parameters and its processing. Study of hybrids and their suitability in an area and micro-area constitutes a permanent field of study to increase the maize production and improving its quality. Based on this principle, a study of ten maize hybrids in western coastal plains of Albania, and specifically in Toshkëz-Lushnja is undertaken.

For the hybrids under study (from Italy, Serbia, Albania) production indicators (, number of rows, number of grains in row and ear, production per plant and grain yield) were evaluated. Yield and yield components were evaluated in ten maize hybrids originated from Italy, Serbia and Albania.

**Keywords:** Maize, hybrid, morphology, yield components, yield.

**Introduction**

The maize is the most prevalent crop in the world. It is considered a green mine, because for a short time, 100-135 days, it gives a dry mass production (grain and green mass), that can't be completed by any other plant. It has high and diverse values of use. Studies in maize plants are numerous, and they continue for different aspects: genetics, breeding, improving of technological parameters and its processing. Study of hybrids and their suitability in an area and micro-area constitutes a permanent field of study to increase the maize production and improving its quality. Based on this principle, a study of ten maize hybrids in western coastal plains of Albania, and specifically in Toshkëz-Lushnja is undertaken.

Study of the suitability of hybrids is one of the possibilities of increasing the yield of maize in different ecological zones. In Albania the hybrids from different countries of the World and Europe are been planted. For determining the appropriate hybrids, especially for the coastal plain west of Albania (Myzeqe) is set up field study of ten hybrids with different vegetation length, mainly late. For all hybrids are made measurements for indicators of the ear, the grain and the grain yield. In the end, the final conclusions are drawn, which have defined hybrids with higher performance and more appropriate for this area.

## Material and methods

The scope of work was to evaluate the biological and the production indicators of some maize hybrids in terms of Western Plain of Albania. As a basic material in this research has been used a eight foreign hybrids and two hybrids synthesized by the ex-Institute of maize and rice in Shkodra, namely:

Experimental Institute of wheat and maize, Bergamo (Italy):

H 605, H 606, H 702.

The Maize Research Institute Zemun Polje (Serbia): ZP 606, ZP 666, ZP 684, ZP 718B, ZP 735.

Ex-Institute of Maize and Rice Shkodra (Albania): R- 683, R-685.

The study was conducted in the village- Toshkez (Lushnja district), in the land of Artan Sota (land owner), in 2011. The land where the experiment was set up is of sub-clay composition with deep subsoil. The pre-crop was wheat.

The soil characteristics are: pH 7.55, humus 2.7%, 0.18% nitrogen, phosphorus 17.7 ppm, 12.5 ppm potassium, calcium 9.07 ppm. The experiment was set up according to the randomized block scheme, with four repetition and ten variants. The elemental plot of each variant was 18 m<sup>2</sup>.

The biometric indicators of ear and production indicators are measured:

### *A 1. The biometric indicators of spikes ear*

The row number in a spike ear

The grain number in a row per row

The grain number in a spike number of grain in ear /per ear

The ear thickness

The cob thickness

The cob weight

### *A 2. The biometric indicators of production*

The weight of ear grains

. 1000 grain weight

The yield /ha.

There are predetermined 20 plants in 4 replications, in which are made biometric measurements

## Results and discussion

Analysis of data for indicators of the ear occupies an important place, Table 1. Ear indicators are important elements of production, therefore are been treated carefully. According to the methodology, the attention was focused on the ear dimensions: thickness, number of rows, number of grains in a row, the number of grains in the ear, the , thickness of cob, thickness of ear. From the data it can be seen that hybrids are characterized by a proven variation. From examination of the data is resulted that, with smaller numbers of rows in the spike is presented hybrid ZP 684 with 14.2 rows, with the largest number is presented R 685 hybrid, with

18.9rows. The number of row and the number of grains per row are two of the key elements of the production of maize, so referred as a selection and evaluation object. The number of grains per row varies from 38.3 for hybrids ZP735 to 45.3 in hybrids H605, average 40.87.

**Table 1. The parameters of ear**

No.	Hybrids	Origin	Number of grains per row	Number of grains per ear	Thickness of ear(cm)	Thickness of cob (cm)	
1	R-683	ALB	43.2	741.1	5.1	3.0	
2	R-685	ALB	40.1	708.7	5.3	3.1	
3	H 605	ITA	45.3	704.6	5.2	3.1	
4	H 702	ITA	39.6	628.8	5.1	3.1	
5	ZP 684	SRB	39.9	566.2	4.8	2.9	
6	ZP 735	SRB	38.3	617.1	5.2	3.1	
7	H 606	ITA	40.3	685.2	5.1	3.0	
8	ZP 718B	SRB	41.1	664.5	5.0	2.9	
9	ZP 606	SRB	41,3	599,3	5.0	2,9	
10	ZP 666	ITA	39.7	677,6	4,9	2,8	
<b>Average</b>			<b>40,87</b>	<b>659,31</b>	<b>5,07</b>	<b>2,99</b>	

The number of grains per ear generally ranges from 600 to 700 grains. per ear in The smallest number of grains per ear has hybrid ZP 684,566.2 grains, and the largest number has hybrid R 685,741,1 grains, averaged 659,31.. The thickness of the ear is a valuable indicator, too. The ear thickness for studied hybrids varies from 4.8 cm for hybrid ZP 684, to 5.3 for hybrid R685. Average thickness of ear for all hybrids is 5,07 cm.

. The minor differences for cob thickness in analysed hybrids (form 2,8 cm in hybrid ZP666 to 3.1 cm in hybrids R685, H605, H702 and ZP735) indicate that no proven differences.

With interest is the study of biometric indicators of grain, Table 2. In this respect were studied two indicators, the weight of grains per ear and weight of 1000 grains. From the data we can observe that there is significant variance among hybrids. Specifically, the weight of grains per ear ranges from 214.5 g for hybrid ZP 684, up to 248, 8 g at hybrid ZP 666. The average weight of grains per ear for tested hybrids is 245.7 g (DMV 0:05 = 18.75 and 0:01 = 22:47).

The weight of 1000 grains is an important indicator as it has to do with the size of grains, which is related to the production. From the data we observe that hybrids represent a wide variation between them. Specifically, this indicator varies from 297 g at hybrid R-683, in 387 g in hybrid.ZP 735 The average weight of 1000 grains for studied hybrids is 356 g.



**Table 2. The data of grain, cob weight and their ratio**

Nr.	Hybrids	Origin	Weight of 1000 grains (g)	Weight of grains per ear(g)	Weight of cob (g)	Grain/cob ratio
1	R-683	ALB	297	224,0	43,1	84:16
2	R-685	ALB	327	230.4	44.1	84:16
3	H 605	ITA	357	259.8	55.1	82.5:17.5
4	H 702	ITA	348	231.2	61	79:21
5	ZP684	SRB	370	214.5	47.6	82:18
6	ZP 735	SRB	387	233.2	48.4	83:17
7	H 606	ITA	342	244.3	44.8	84.5:15.5
8	ZP 718B	SRB	378	242.6	54	81.8:18.2
9	ZP 606	SRB	380	238,7	45,1	84:16
10	ZP 666	SRB	378	248,8	40,3	86:14
<b>Average</b>			<b>356,4</b>	<b>236,75</b>	<b>48,35</b>	<b>83:17</b>

It is known that the ear is consisted of grains and the cob where the grains are been placed. At the overall weight of ear is intended that cob weight to be as small as possible. Usually, the ratio grain/cob is expressed in percentage. The cob weight ranges from 43.1 g at hybrid R 683, to 61 g at hybrid H 702. The average weight of cob to the studied hybrids is 48,35 g.

Ratio grain/cob represents small differences, where the best value was at ZP 666 hybrid (86:14); the value has diminished at ZP 718 B hybrid (81,8 : 18,2).

It is understood that in a comparative study of hybrid performance, the main indicator and the most important is, undoubtedly, yield. Hybrids represent various differences in performance. In this case, hybrids are grouped by production capacity starting from the highest to the one with the lowest.

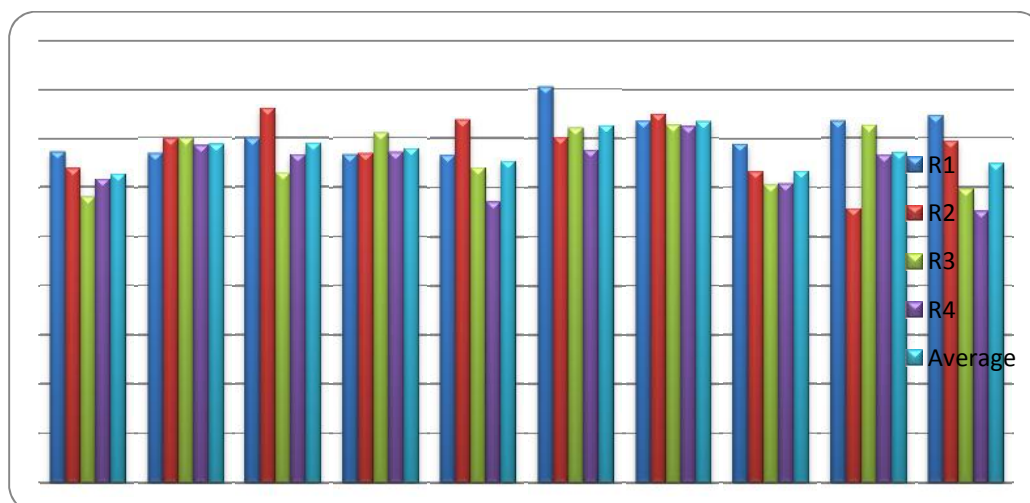


Figure 1. Yields data ( t/ha) P. 0.05=10.4 q/ha P. 0.01= 13.54 q/ha

Hybrids represent a significant variance for yield, Fig.1. Specifically, it varies from 127 875 t/ha to ZP 718B hybrid, to 150.1 q / ha to PR31A34 hybrid. It appears from the analysis of variance tables, in which significant variations through hybrids are been observed, where  $f$ -factual is greater than  $f$ -critical.

### Conclusions

For all biometric indicators of ear and grain there are differences between hybrids, which materialized to achieve production for each hybrid. Referring to the performance indicators, hybrids can divide into three groups:

The first group hybrids: H 606,ZP 735, R 685, H 605, which provide the highest performance for both levels of security.

The second group: ZP 606 and H 702, which provide the highest performance for one security level (0, 01).

The third group: R- 683, ZP 684, ZP 718 B, ZP 666 that don't have good results.

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**PRODUCTION AND BIO-ACTIVE POTENTIAL OF OLD TOMATO CULTIVARS  
ORIGINATING FROM FORMER YUGOSLAVIA**

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**Abstract**

Two old tomato cultivars (*Sarajevski jabucar* and *Novosadski jabucar*) have been studied for the production and bio-active potential. Investigation was performed in Burmir, Sarajevo, BH. The research included fruit weight and the yield of the fresh fruits per hectare. Lycopene was found in fresh fruits both in physiological and technological maturity. Fruit weight was significantly higher for *Sarajevski jabucar* than for *Novosadski jabucar*. Lycopene level in *Sarajevski jabucar* in physiological maturity was from 6.20 to 9.95, and in technological from 8.35 to 13.60 mg per 100 grams of fruit. Old varieties that have become domestic in the Balkan belong to genotypes rich in lycopene. The production potential was at the satisfactory level in some growing areas.

**Keywords:** tomato, old varieties, yield, level of bioactive matters

**Introduction**

Physiologically ripe tomato fruit, its refreshing, sweet-sour taste and the biological value contributed to the widespread use in diet. In European countries, 5-10% of the total consumption of vegetables is the tomato. This vegetable has an important place in the diet because of its culinary and nutritional properties. Tomatoes are especially valuable due to the contents of lycopene, which affects the colour of the fruit (Ili et al., 2012) and acts as an antioxidant. Numerous epidemiological studies have confirmed the role of lycopene as a micronutrient with favourable effects on health (Markovi , 2006).

The main function of lycopene is to protect the cell and its structure from the destructive effects of free radicals, which is extremely important in the prevention of many diseases. Lycopene is the most powerful antioxidant from the carotenoid group. It is a powerful deactivator of reactive oxygen forms. Activity of lycopene in slowing the progression of the cell cycle partly explains the slowdown in the spread of certain types of cancer. It can prevent malignant transformation - cellular process that represents the transformation of normal cells into cancer cells. It restores communication channels between cells and helps eliminate carcinogenic substances and toxins from the body. When combined with other natural antioxidants, it slows the oxidation of LDL cholesterol, which is a key factor in the development of atherosclerosis and coronary heart disease. More than 85% of lycopene comes from tomatoes and tomato products. The use of tomato products is recommended to ensure the intake of 40 mg of lycopene, which is sufficient to reduce LDL cholesterol and the development of arteriosclerosis and heart disease. (Rao and Agarwal, 1999). The main task of antioxidants is to protect the body from oxidative stress and condition in which there is an

imbalance between free radicals and antioxidants that fight against them. Antioxidant functions of the lycopene are associated with decrease of DNA damage, malignant transformation and biological reduction of biological oxidative damage of proteins, lipids and other cell parts (Shi and Le Maguer, 2000).

The content of lycopene in fresh tomato fruit ranges from 85 to 181 mg/kg. During three growing seasons, lycopene content was different for the same varieties and ranged from 88, 101 to 106 mg/kg (Garcia and Barrett, 2006). Results of the previous researches proved that the concentration of lycopene depends on the vegetation season, location, variety and degree of ripeness. Besides genetic factors, the content varies depending on crop management, especially depending on the input of nutrients into the soil (nitrogen, phosphorus, potassium and calcium) and temperature variations (Dumas et al., 2003; Adeniyi and Ademoyegun, 2012). The content of lycopene is genetically determined, but is strongly influenced by environmental conditions. Lycopene content can be changed by reducing or increasing the amount of potassium (Zdravkovic et al., 2001; Zdravkovic et al., 2007). Agronomic aspects of irrigation i.e., reduced irrigation treatments and acceptable yield, greatly alter or increase the dry matter content of the fruit, and thus increases the lycopene content (Savic et al., 2004). The level of lycopene is also different depending on the place of growing: an open field or protected area (Jarquín-Enríquez et al., 2013; David et al., 2013). Growing season can affect different lycopene content in fruits (Assimakopoulos, 2010). Recently, differences and variations in the lycopene content in tomato grown in organic systems versus the conventional way of farming was studied (Riahi et al., 2009; Barrios-Masias et al., 2011; Riahi and Hdidier, 2013).

The aim of this study was to determine the lycopene content as an antioxidant and yield of two tomato varieties originating from the former Yugoslavia (*Novosadski* and *Sarajevski jabucar*), which is now cultivated in large areas. High quality and lycopene content of these varieties involves them in the selection of varieties that are intended for organic production at the Balkan Peninsula, where these old varieties are traditionally being grown.

### **Materials and Methods**

The study was carried out on the site: Butmir (Sarajevo) (cca 500 m, M.S.L), in years 2010 and 2011, on brown valley soil. Features: fruit weight, yield of fresh fruit per acre of the old varieties: *Novosadski jabucar* and *Sarajevski jabucar* were tested. Chemical properties were investigated on fresh fruits, while lycopene was detected in fresh fruits and in tomatoes juice. The tests were performed according to methodology of testing new and foreign tomato varieties issued by the Federal Ministry of Agriculture, Water Management and Forestry (2006.) in random block system with 5 replications.

The number of plants in the experiment was 100%. Irrigation was performed by drip system. Studied plants reached the stage of five flowers. After this stage the plant top was cut off. Measurements were taken in six harvests. During the study, tomato fruit samples were taken for determination of the bioactive properties of variety *Vra ar* (2001):

Dry matter (%) – by drying at 102-105<sup>0</sup>C

The degree of acidity (%) – titrimetrically

Total sugars – according to Luff-Schoorl gravimetrically

Lycopene – spectrophotometrically (Beerh and Siddappa, 1959)

Results were analyzed by applying analysis of variance method. Differences among the varieties were expressed by the least significant difference (LSD test).

**Agro-ecological conditions of cultivation**

Table 1. Chemical traits of soil (location: Butmir, B&amp;H)

Year	pH H <sub>2</sub> O KCl		Total N %	Humus %	P <sub>2</sub> O <sub>5</sub> mg / 100g	K <sub>2</sub> O <sub>2</sub> mg / 100g
2010	6,02	-	0,08	1,80	8,45	14,20
2011	6,28	5,45	0,230	1,75	11,50	8,50

Soil in 2010 and 2011 was poorly supplied with nitrogen and potassium. The land had a higher content of phosphorus in 2009 than in 2010.

Table 2. Average monthly air temperature (°C) for 2010 and 2011 (location Butmir, B&amp;H)

Year	I	II	III	IV	V	VI	VII	VIII	IX
2010	0,4	1,8	5,2	10,4	14,4	18,1	20,8	20,9	15,0
2011	0,2	0,6	5,2	11,0	14,0	18,9	20,5	21,7	19,1
Annual average (1996-2005)	0,07	1,02	5,27	9,58	15,16	18,7	20	14,4	11,1

Table 3. Maximal monthly air temperature (°C) in 2010 and 2011 (Location: Butmir, B&amp;H)

Year	I	II	III	IV	V	VI	VII	VIII	IX
2010	15,4	14,2	22,4	25,2	28,0	34,4	34,9	20,9	15,0
2011	13,9	14,4	22,5	24,9	29,8	33,2	37,9	37,5	34,5
Annual average (1996-2005)	16,7	19,6	26,6	29,8	32	34,2	38,2	37,4	30,8

Table 4. Minimal monthly air temperature (°C) 2010 and 2011

Year	I	II	III	IV	V	VI	VII	VIII	IX
2010	-11,4	-13,5	-9,5	1,4	5,6	5,7	9,4	20,9	15,0
2011	-9,9	-10,2	-10,3	0,1	0,8	7,6	9,1	8,9	6,7
Annual average (1996-2005)	-21	-16,4	-16,8	-6,2	0,5	2,5	5,4	5,5	2,8

Table 5. Monthly precipitation (l/m<sup>2</sup>) in 2010 and 2011

Year	I	II	III	IV	V	VI	VII	VIII	IX
2010	161,3	95,9	63,1	59,1	93,2	181,5	19,2	20,9	15,0
2011	37,9	35,9	40,7	32,7	103,6	76,3	134,4	4,8	38,9
Annual average (1996-2005)	70	71,4	50,8	85,1	70,6	71,8	74,5	65,3	124

Temperatures during this study (2010 and 2011) were in the range of annual average.

### Results and discussion

Table 6. Fruit weight (g)

Characteristic	<i>Sarajevski jabucar</i>		<i>Novosadski jabucar</i>	
	2010.	2011.	2010.	2011.
Fruit weight (g)	184	254	93	88

Fruit number and fruit weight were varietal characteristics. Varieties with large fruits have a lower number of fruits per plant and in inflorescence, and vice versa (Taka , 2001).

*Sarajevski jabucar* had higher fruit weight comparing to *Novosadski jabucar*, during two years of research. The average fruit weight was from 88 to 93 grams for *Novosadski jabucar*, which was less than results obtained by (Krsti , 2006) in Vojvodina growing conditions. Fruit weight of *Sarajevski jabucar* was from 184 (2010) to 254g (2011), Table 6. Great variation of fruit weight within a genotype which is a variety bred from the population is in accordance with results obtained by Zdravkovi (1997). She researched old varieties that varied a lot within the genotype.

Table 7. Impact of factors of variety and year on the yield (t/ha)

Variety	Year				Average yield t/ha
	2010.		2011.		
	t/ha	Rel.	t/ha	Rel.	t/ha
<i>Sarajevski jabucar</i>	47,22**	157	30,84**	130	39,03*
<i>Novosadski jabucar</i>	29,92	100	23,68	100	26,80
<i>LSDp=5%</i>	2,24		0,79		8,84
<i>LSDp=1%</i>	3,52		1,127		14,62

Yield of *Sarajevski jabucar* were significantly higher in 2010 and 2011 comparing to *Novosadski jabucar*. In 2010 yield of *Sarajevski jabucar* was 57% higher, while in 2011 it was 30% higher comparing to *Novosadski jabucar*. Biological potential of some plant species and varieties for synthesis of organic matters is genetically controlled (Sari , 1984).

Table 8. Chemical composition of tomato fruits per harvest

Physiological maturity of the fruit	Date of yield	<i>Sarajevski jabucar</i>	<i>Novosadski jabucar</i>
Dry matter (%)	9.8.2010.	4,98	5,73
	26.8.2011.	4,65	6,05
Total sugars (%)	9.8.2010.	3,85	4,80
	26.8.2011.	3,30	4,08
Acidity, expressed as acetic (g/100 ml of product)	9.8.2010.	0,45	0,27
	26.8.2011.	0,33	0,43
Lycopene (mg/100 g)	9.8.2010.	-	-
	26.8.2011.	9,81	9,50

Chemical-technological analysis of tomato samples gave: % of dry matter, % of total sugars and acidity expressed as acetic in 2010 and 2011, and lycopene in 2011. *Sarajevski jabucar* had lower percentage of dry matter and total sugars in 2010 than in 2011. *Novosadski jabucar* had higher % of dry matter in 2011, and higher % of total sugars in 2010. Acidity in fruits

expressed as acetic acid was lower in *Sarajevski jabucar* than in *Novosadski jabucar* in 2011, while it was vice versa in 2010.

The acid content greatly affects the technological value of tomatoes. The high acidity (low pH) determines the mode of conservation of various tomato products (pasteurization, not sterilisation like other vegetables). Acid content in tomatoes varies depending on the variety and maturity (Krstić, 2006).

Table 9. Percentage of dry matter, total sugars, acids and lycopene per harvest in Butmir - Sarajevo

Date of harvest	Old cultivar	Dry matter %	Total sugars %	Total acids %	Lycopene (mg/100g )	
					Physiological maturity	Technological maturity
26.08.2011.	<i>Sarajevski jabucar</i>	<b>4.65</b>	<b>3.30</b>	<b>0.33</b>	<b>9.81</b>	<b>10.95</b>
26.08.2011.	<i>Novosadski jabucar</i>	<b>6.05</b>	<b>4.08</b>	<b>0.43</b>	<b>9.50</b>	<b>10.63</b>
02.09.2011.	<i>Sarajevski jabucar</i>	<b>5.36</b>	<b>3.83</b>	<b>0.50</b>	<b>9.95</b>	<b>11.15</b>
02.09.2011.	<i>Novosadski jabucar</i>	<b>6.07</b>	<b>4.32</b>	<b>0.53</b>	<b>9.74</b>	<b>10.05</b>
09.09.2011.	<i>Sarajevski jabucar</i>	<b>5.01</b>	<b>3.06</b>	<b>0.24</b>	<b>8.50</b>	<b>9.95</b>
09.09.2011.	<i>Novosadski jabucar</i>	<b>6.67</b>	<b>4.70</b>	<b>0.28</b>	<b>6.76</b>	<b>8.23</b>
16.09.2011.	<i>Sarajevski jabucar</i>	<b>4.66</b>	<b>2.66</b>	<b>0.37</b>	<b>7.40</b>	<b>8.35</b>
16.09.2011.	<i>Novosadski jabucar</i>	<b>6.60</b>	<b>4.40</b>	<b>0.46</b>	<b>6.15</b>	<b>7.30</b>
21.09.2011	<i>Sarajevski jabucar</i>	<b>5.19</b>	<b>3.12</b>	<b>0.38</b>	<b>6.20</b>	<b>9.54</b>
21.09.2011.	<i>Novosadski jabucar</i>	<b>5.91</b>	<b>3.36</b>	<b>0.62</b>	<b>5.84</b>	<b>9.85</b>
27.09.2011-	<i>Sarajevski jabucar</i>	<b>6.04</b>	<b>3.84</b>	<b>0.30</b>	<b>7.56</b>	<b>13.60</b>
27.09.2011.	<i>Novosadski jabucar</i>	<b>5.97</b>	<b>3.80</b>	<b>0.33</b>	<b>6.80</b>	<b>9.20</b>

*Novosadski jabucar* had higher level of dry matter, total sugars and total acids.

Balacheva et al. (2011) determined by biochemical analyzes, no significant differences between the genotypes of yellow and red fruit in the content of dry matter and sugar. The moment of harvest, a stable survival of lycopene and other biochemical parameters in stages after picking are essential for selecting genotype (Brashlyyanova and Ganeva, 2009).

Lycopene content was higher in fruits of *Sarajevski jabucar*. Lycopene content was higher in technological than in physiological maturity. Changes in lycopene level increase or decrease antioxidative impact and it is desirable to find a tomato genotypes with high content of lycopene in fruit (Ilahy et al., 2011). Lycopene level in *Sarajevski jabucar* in physiological maturity ranged from 6.20 to 9.95, and in technological maturity from 8.35 to 13.60 mg/100

grams of fruit. In studies of Zdravković et al. (2002) lycopene levels were significantly lower than in our tests. The researched genotype *Novosadski jabucar* had the average value of 5.6 mg%, which is not in accordance with the results of our study. The level of lycopene in *Novosadski jabucar*, in physiological maturity ranged from 5.84 to 9.74, and in technological maturity from 7.30 to 10.63 mg/100grams of fruit. Lycopene level varied depending upon variety and yield. The degree of expression of lycopene depends on the time of maturity. These tests included the 6 harvests, so the level of lycopene was steady because the fruits were at the same stage of maturation (Riggi et al., 2008). With ripening, the level of lycopene is changing and growing, even in the post-harvest period. Tomato fruits with climacteric breathing are able to develop normal colouring after harvesting and the synthesis of the pigments continues during ripening (Ilić et al., 2007).

Reduction of total acid and lycopene in heterozygous *rin/rin+* F1 tomato hybrids was not statistically significant. There were no statistically significant difference among hybrids and lines in chemical compound, except for the level of lycopene. According to Maković et al. (2010) mother components of hybrids (*rin/rin* genotype) have low level of lycopene (6,0-6,8 mg%) comparing to hybrids with significantly higher level of lycopene (13,2-22,4 mg%).

Results of the analysis of tomato juice on the content of lycopene obtained in Butmir 2011 were similar to results obtained by Marković (2002). The content of lycopene ranges in varieties of pink and red fruits from 3.7 to 5.9 mg (Balacheva et al., 2011).

### Conclusion

Old and domesticated tomato varieties grown in the Western Balkan belong to the tomatoes that are rich in flavour and aroma, with satisfactory yield and acceptable level of resistance to diseases. These genotypes represent good start selection material for breeding varieties and hybrids intended for this market, where the consumers are used to tomato of well defined taste and aroma and, on the other hand, well adapted to ecological conditions of Balkan.

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## YIELD AND MINERAL COMPOSITION OF TWO NEW ONION VARIETIES FROM BOSNIA AND HERZEGOVINA

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### Abstract

Onions are grown for a variety of purposes. The purpose of the production determines the choice of variety and growing technology. In this paper, the Federal Institute for Agriculture in Sarajevo introduces two new varieties-Konjica's and Zenica's onion. The new varieties are specially developed for production from onion sets to suit the environmental conditions of Bosnia and Herzegovina. This paper evaluates the new varieties production characteristics and their bulb mineral composition, tested in Butmir (Sarajevo municipality) over 2009 and 2010 with the Stuttgarter cultivar used as a standard. The new varieties are medium late, the plants are well developed and have erect leaves that are dark green in color and have a pronounced waxy coating. The new onion varieties significantly out-yielded the standard cultivar. Over two years, Konjica's onion yielded 30.68 t ha<sup>-1</sup> on average (51% more than Stuttgarter cultivar), while Zenica's onion yielded 24.83 t ha<sup>-1</sup> on average, 21% more than the standard cultivar. The mean concentrations of trace element in the bulbs of the new varieties were in the order: Zn>Fe>Mn>Cu indicating onions from Bosnia and Herzegovina as a good source of various vitamins and minerals important to maintain human health.

**Keywords:** onion varieties, yield, mineral composition

### Introduction

Vegetable consumption helps meet the body's needs for minerals, vitamins, dietary fiber and in a small way, for protein. Nutritionally, vegetables are excellent sources of carotene, folate, niacin, iron, Vitamin C and calcium. These are of special importance in the prevention of some forms of cancer, heart disease, stroke and other chronic diseases.

Onions are one of the most versatile vegetables, used year round, either fresh (green onions, mature bulbs), or processed (dehydrated, pickled, canned). Like other vegetable species, onions provide vitamins and a good amount of minerals to the human body. Choice of cultivar and proper cultural practices are important facets in the production process. Choosing the right cultivar is one way of ensuring high yields and good quality of onion. The purpose of an onion determines the choice of variety and growing technology used. Hence the goals of onion breeding programs vary (Gvozdanović-Varga *et al.*, 1996). Onions may be grown from seed, transplants or seed-grown sets (sets started from seeds from the previous year). Cultural system is determined by the cultivar's biology, environmental conditions and food preferences in the locality (Lazić *et al.*, 2000). Yields are highest where the crop is grown directly from seed and modern growing technologies are fully utilised, whereas yields are low where onions are grown from sets (Gvozdanović-Varga *et al.*, 2005). In Bosnia and

Herzegovina, onion is extensively cultivated and production of the crop from sets is predominant. Therefore, the Federal Institute for Agriculture in Sarajevo has specifically developed two new onion varieties, Konjica's onion (Konji ki) and Zenica's onion (Zeni ki) for production from onion sets that will suit the environmental conditions of the country.

The paper evaluates the varieties's production characteristics and their bulb mineral composition, tested in Sarajevo (Butmir) over two consecutive years with the Stuttgarter cultivar used as a standard.

### Material and methods

Variety evaluation trials were conducted in Butmir (43°49 N 18°20 E) in the Sarajevo Canton of Bosna and Herzegovina in 2009 and 2010. Cultivar Stuttgarter, commonly grown and cultivated from sets in Bosnia and Herzegovina was used as the standard.

Before the trials were set up, average soil samples were taken and chemical analyses were carried out. The soil was of the brown valley type, poorly supplied with phosphorus and moderately supplied with potassium across both years (Table 1.). In accordance with soil tests and commercial recommendations, 56, 112, and 294 kg ha<sup>-1</sup> of Nitrogen, Phosphorus and Potassium was applied respectively every year. The trials were conducted in a randomised block design with five replicates. Plot size was 6 square meters. March 30<sup>th</sup> 2009 and 2010 were the sowing dates. Seeds were planted manually in triple rows spaced 30 cm apart and within-row spacing 10 cm resulting in population density of 150 plants per plot. Soil management, pest and disease control was carried out according to standard procedures. The date of technological maturity was recorded. After the onions were lifted and dried, production characteristics (yield and days to maturity) and their bulb mineral composition at full maturity were determined. Bulb mineral content was determined according to EN 13805:2002, ITD and EN 15763:2009, ITD method using Induced coupled plasma with mass spectrometry (ICP-MS 7700x, Agilent Technologies, Japan) using plant material previously prepared in a microwave oven for digestion (MDS-8, Sineo, China). Plant material samples were destroyed with Nitric acid 65% Suprapur purity, Hydrogen peroxide, 30% Suprapur purity and Hydrochloric acid 30% purity Suprapur. Detection limits were: Cr-0.99 ng kg<sup>-1</sup> (ppt), Mn-0.66 ppt, Fe-9.17 ppt, Co-0.14 ppt, Cu-0.41 ppt, Zn-1.02 ppt, Mo-0.12 ppt, Pb-0.16 ppt.

Data (minimal, maximal and mean monthly temperatures and monthly precipitation sums) from the weather station at Butmir were used to obtain weather data for the growing seasons studied. Yield data were analysed for the differences between the standard (Stuttgarter) and the tested varieties (Konji ki and Zeni ki) using analysis of variance. Mean separation was based on a LSD test. Differences were considered to be significant at  $P=0.05$  and  $P=0.01$ .

Table 1. Soil composition in Butmir, Bosnia and Herzegovina.

Year	pH		Content (%)			Physiologically active (mg 100g <sup>-1</sup> of soil)	
	in H <sub>2</sub> O	in KCl	Total N	CaCO <sub>3</sub>	Humus	P <sub>2</sub> O <sub>5</sub>	K
2009	5.89	-	0.09	-	1.80	12.50	10.90
2010	6.02	-	0.08	-	1.80	8.45	14.20

### Results and discussion

Growing conditions during variety evaluation trials

Seed-grown sets make a more robust plant at emergence than seeds, have a shorter growing

season and the whole plant growth is less dependent on conditions of environment (Brewster, 2008; urovka, 2008). If higher temperatures prevail in germination stage, rooting may be shortened and above-ground grow hastened ( urovka, 2008).

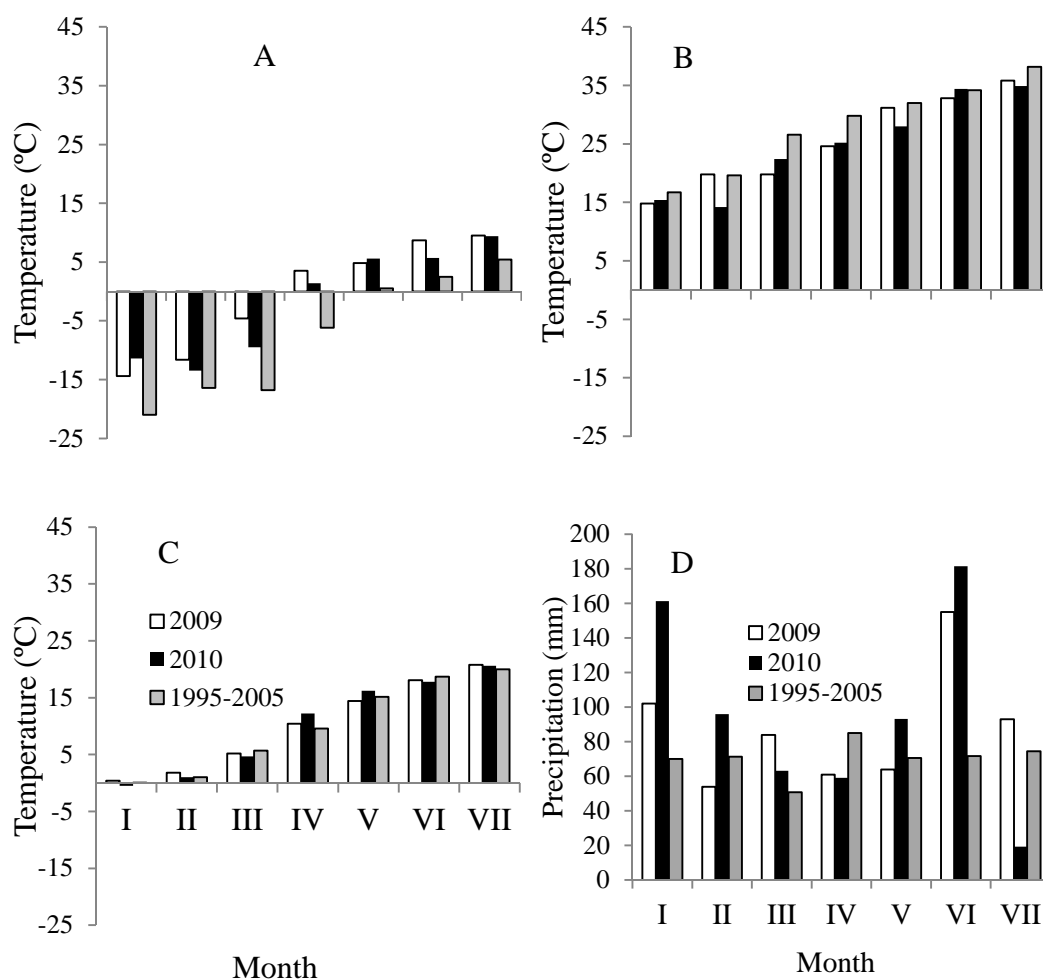


Figure 1. Weather conditions in onion growing season. Panel A, minimal monthly air temperatures. Panel B, maximal monthly air temperatures. Panel C, mean monthly air temperatures. Panel D, monthly precipitation sums.

During trial years, maximal and mean monthly air temperatures in Butmir were within the long-term average range (Figure 1B and 1C). After planting and April temperatures in 2009 and 2010 were within range of optimal 5-10 °C and 10-15 °C for growing stages rooting and germination, respectively. Therefore, well developed above-ground plant parts and a strong rooting system secured good bulb formation. According to Jones and Mann (1963), under conditions of extremely high temperatures, bulbs will mature early and yields may be reduced; at low temperatures, maturity is usually delayed, curing becomes difficult, and storage quality is often impaired. In 2009 and 2010 in Butmir during the stage of bulb formation (late April and early May), mean monthly air temperatures though lower than the optimal for the stage (22 °C) (Lazić *et al.*, 2001) were higher than nine-year average so both varieties obtained good yields.

Irrigation during leaf and early bulb growth ensures that onion crop reaches a high LAI (Leaf Area Index) as quickly as possible. This will tend to promote rapid ripening and good bulb quality for storage (Brewster, 1990). In Butmir wetter-than-average conditions were observed

in January, March and June in 2009 and 2010 (Figure 1D). April in both seasons was unusually dry. Compared to the multiyear average (74.5 mm) July of 2010 was extremely dry (19.2 mm). Irrigation late in bulbing may delay maturity and reduce bulb quality by virtue of skin splitting and rotting, particularly if they follow a period in which growth has been restricted by lack of water or Nitrogen nutrition (Brewster, 1990).

#### Cultivars agronomic performances

The new varieties, Konji ki and Zeni ki, are medium late (in both years technical maturity reached in 114 days), just as the standard is (data not shown). The plants are well developed and have erect leaves that are dark green in color and have a pronounced waxy coating.

Table 2. Yield of Stuttgarter, Konji ki and Zeni ki variety in 2009 and 2010. Asterix indicates significant differences at  $P=0.05$  and \*\* is significant at  $P=0.01$  by LSD test.

Variety	Year			
	2009		2010	
	Yield (t ha <sup>-1</sup> )	%	Yield (t ha <sup>-1</sup> )	%
Stuttgarter	22.88	100	18.0	100
Konji ki	37.36**	163	24.0**	134
Zeni ki	29.16**	127	20.5**	114
LSD <sub>P=5%</sub>	1.16		1.57	
LSD <sub>P=1%</sub>	1.70		2.29	

In both trial years, the new varieties significantly out-yielded the standard variety (Table 2). Tested varieties yielded lower in 2010 (Konji ki 24.00 t ha<sup>-1</sup>, Zeni ki 20.5 t ha<sup>-1</sup> and Stuttgarter 18.0 t ha<sup>-1</sup>), 13.26 t ha<sup>-1</sup>, 8.66 t ha<sup>-1</sup> and 4.88 t ha<sup>-1</sup> less than in 2009, respectively. Over the two years, the new tested variety-Konji ki obtained an average yield of 30.68 t ha<sup>-1</sup>, or 51% more than standard variety- Stuttgarter (20.44 t ha<sup>-1</sup>) (Table 3). The tested variety Zeni ki over the two years out-yielded the standard variety for 21% (Table 3).

Yield varieties in 2009 were significantly higher than in 2010 (Table 4). Temperature and rainfall conditions, in contrast to day-lengths, are never exactly alike, year after year, in the same district; consequently, onion cultivars cannot be expected to perform consistently, even though cultural practices are similar (Jones and Mann, 1963).

Table 3. Effect of variety on average yield (2009-2010). Asterix indicates significant differences at  $P=0.05$  and \*\* is significant at  $P=0.01$  by LSD test.

Variety	Average yield (t ha <sup>-1</sup> )	%
Stuttgrater	20.44	100
Konji ki	30.68**	151
Zeni ki	24.83**	121
LSD <sub>P=5%</sub>	0.80	
LSD <sub>P=1%</sub>	1.08	

Table 4. Effect of year on onion yield. Asterix indicates significant differences at  $P=0.05$  and \*\* is significant at  $P=0.01$  by LSD test.

Year	Yield in t ha <sup>-1</sup> (average for all three varieties)	%
2009	29.8 <sup>**</sup>	143
2010	20.83	100
LSD $P=5\%$	0.65	
LSD $P=1\%$	0.88	

#### Bulb trace element composition

The mean concentrations of trace element in the bulbs of the new varieties were in the order: Zn (1.313 mg kg<sup>-1</sup>) > Fe (0.72 mg kg<sup>-1</sup>) > Mn (0.324 mg kg<sup>-1</sup>) > Cu (0.221 mg kg<sup>-1</sup>) > Cd (0.015 mg kg<sup>-1</sup>) > Pb (0.003 mg kg<sup>-1</sup>) > Cr (0.003 mg kg<sup>-1</sup>) (data not shown). Highest Mn (0.436 mg kg<sup>-1</sup>) and Cd (0.03 mg kg<sup>-1</sup>) content was determined in bulbs of Stuttgrater variety, Fe (1.145 mg kg<sup>-1</sup>) and Cu (0.244 mg kg<sup>-1</sup>) in bulbs of Konji ki and Zn (1.715 mg kg<sup>-1</sup>) in bulbs of Zeni ki variety (Table 5). The average Cu, Zn, Pb and Cd content was similar of 15 onion populations from Vojvodina Province (0.204-0.818 mg Cu kg<sup>-1</sup>; 0.452-2.025 mg Zn kg<sup>-1</sup>) (Maksimovic *et al.* 2012).

According U. S. Department of Agriculture (2003) intake of 100g of onions provides 0.04 mg of Cu, 0.19 mg of Fe, 0.16mg Zn and 0.13mg of. Results indicate onions from Bosnia and Herzegovina as a good source of various vitamins and minerals important to maintain human health. Intake of 100 g of tested varieties provides 55% of necessary daily amount of Cu, 126% of Fe, 82% of Zn and 83% of Mn recommended by the U. S. Department of Agriculture (2003).

Table 5. Trace element composition of the onion bulbs (mg kg<sup>-1</sup>). Trace element content presented as nd is not detected.

Trace element	Variety		
	Konji ki	Zeni ki	Stuttgrater
As	0.0004	0.0004	0.001
Cr	0.005	0.004	0.001
Cd	0.007	0.007	0.03
Mn	0.194	0.344	0.436
Fe	1.145	1.071	0.943
Co	0.0002	nd	0.001
Cu	0.244	0.186	0.233
Zn	1.158	1.175	1.065
Mo	nd	nd	nd
Pb	0.003	0.002	0.006

#### Conclusions

Two new varieties Konjica's onion (Konji ki) and Zenica's onion (Zeni ki) suit the environmental conditions of Bosnia and Herzegovina. They are medium late, the plants are well developed and have erect leaves that are dark green in color and have a pronounced waxy coating and significantly out-yielded the standard cultivar. The mean concentrations of trace element in the bulbs of the new varieties were in the order: Zn > Fe > Mn > Cu indicating onions from Bosnia and Herzegovina as a good source of various vitamins and minerals

important to maintain human health. Expansion of domestic varieties of high yield and good quality such as Konji ki and Zeni ki, development of varietal technology and controlled onion set production should result in an increase of domestic production of the crop and reduction of imports from abroad.

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## POMOLOGICAL PROPERTIES OF „GALA“ APPLE CLONES IN THE REGION OF SARAJEVO

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### Abstract

The paper presents results of two – year study of some pomological properties of four clones of apple cultivar ‘Gala’ (‘Mondial Gala® Mitchgla’, ‘Gala Schniga® SchniCo(s)’, ‘Gala Must®’, ‘Galaxy’). The research was carried out in the apple orchard for cultivar testing of Federal Bureau of Agriculture of Bosnia and Herzegovina and included the phenological characteristics, physical characteristics of the fruit and yield.

The obtained results have confirmed that agro – environmental conditions of Sarajevo are favorable for growing the above – mentioned clones. All four of ‘Gala’ apple clones has shown good physical properties of the fruit and they can be recommended for commercial growing in the region of Sarajevo. These cultivars may greatly contribute to the advancement of Bosnia and Herzegovina apple assortment.

**Key words:** apple, clones, ‘Gala’, pomological properties.

### Introduction

Apple (*Malus domestica* Borkh.) is one of the most important and most cultivated fruit species both in the world and in our country. The reason for this is the long period of maturation (from early summer cultivars to late winter cultivars) and wide use value (many cultivars are used as fresh fruit, while a number of cultivars are used for processing).

Total production, trade and consumption of apples, is in third place in the world, behind citrus and bananas. During the period 1998 – 2007, the average apple production was 59.855409 t in the world (Nikoli and Fotiri, 2009). The most important producer countries are China (37%), United States (7%), Italy (4%), Turkey (4%), Iran (4%), Russia (3%), and Poland (3%). The production of apples is at the second place with a share of 24.94% of the total fruit production in Bosnia and Herzegovina. According to the data of Federal Bureau of Statistics, there has been an increase in the number of planted and productive trees recently, affecting an enlargement of production of apples. The environmental conditions of Sarajevo are ideal for the development of fruit production, and especially the production of apples. Major problem in production of apple in our country and in the neighboring countries is inadequate and obsolete assortment. In this assortment, mostly dominate the autochthonous and standard cultivars, with cultivar ‘Idared’, which takes the leading place in assortment of apples (Milatovi et al., 2009). This cultivar is characterized by a high yield, large fruit, and good storage capacity, but the big a problem is bad quality of the fruit. At the present time the main goal is to modernize the existing assortment through the introduction of new cultivars.

Most of the cultivars of apples were developed in process of clonal selection. Cultivar that is very prone to mutation is ‘Gala’. According to Hampson and Kemp(2003), cultivar ‘Gala’ belongs to the group of 12 major cultivars of apples, but this cultivar is only sporadically present in our country.

Clones of apple ‘Gala’ differ in additional color, the abundance of blooming, ripening time, yield, fruit size, but quality of fruit does not vary (*Kappel et al.*, 1992; *Greene and Autio*, 1993).

The aim of this work was the study of phenological characteristics, physical characteristics of the fruit, and yield of four clones of apple ‘Gala’ in the region of Sarajevo. According to this results, recommendation of the best clones for commercial growing in the conditions of Sarajevo could be made.

### **Materials and methods**

The research was carried out during 2011 and 2012, on the location of Sarajevo, in the apple orchard for cultivar testing of Federal Bureau of Agriculture of Bosnia and Herzegovina located in Butmir – Ilidza. Test plantation was built in the spring of 2007, at the altitude of 600 meters above sea level. Training system is slender spindle with spacing of planting 3,3×1 m (3030 trees/ha). Four clones of apple ‘Gala’ were studied: ‘Mondial Gala® Mitchgla’, ‘Gala Schniga® SchniCo(s)’, ‘Gala Must®’, and ‘Galaxy’. Rootstock for all clones was M9.

The area in which the orchard is established is characterized submountainous climate (Hydrometeorological Institute of Bosnia and Herzegovina), with cold winters that last longer than in the continental zone. The average annual air temperature is below 10°C. Autumn is warmer than spring. The absolute maximum temperature does not exceed or very rare exceeds 30°C in summer. The minimum temperature does not fall below -30°C in the winter. The annual precipitation ranges from 750 to 1.000 mm, and they are, mostly, unevenly distributed. Snow takes an average of 2 – 3 months. Agro – environmental conditions in which the experiment was set, have lead to the use of standard cultural practices. The research was included the phenological characteristics, physical characteristics of the fruit and yield.

The phenological characteristics included the time of flowering (beginning, full, end and duration of flowering) and the date of harvest. Flowering was followed by recommendations of the International working group for pollination (*Wertheim*, 1996). The date of beginning of flowering was taken when 10% of flowers were open, full – when 80% of flowers were open, end – when 90% of petals were fallen. Duration of flowering was determined by the number of days from the beginning to the end of flowering. The date of harvest is taken as the time of maturing. Characteristics of fruit are determined on a sample of 30 fruits for each clone. Standard morphometric methods were used to determine the weight of fruit, length and width of fruit and index of the fruit shape.

Yield of examined ‘Gala’ apple clones was registered by measuring the the weight of all fruits on the tree. It is expressed in kg/ha and kg/tree for each clone.

The results are processed by the statistical method of the analyses of variance for two factorial experiment. The significance of differences between mean values is determined by Duncan’s multiple range test at  $P=0.05$  (*Dunnnett*, 1955).

### **Results and discussion**

*Phenological properties.* The phenological properties included the time of flowering and the date of harvest. Yield of apple trees depends on beginning, full, end and duration of flowering. The flowering time is greatly influenced by weather, particularly by temperature and relative humidity before the beginning of flowering and during flowering.

Although the beginning of apple flowering is mainly caused by weather conditions, the sequence of flowering of cultivars grown in identical agro – environmental conditions, is caused by the genetical characteristics of the cultivars.

The results of flowering and the time of maturation of examined ‘Gala’ apple clones are presented in Table 1.

Table 1. Phenological properties of ‘Gala’ apple clones in conditions of Sarajevo (average, 2011-2012)

Cultivar	Flowering			Duration /days	Time of maturation
	Beginning	Full	End		
‘MondialGala®Mitchgla’	24.04.	29.04.	07.05.	13	02.09.
‘Gala Schniga®SchniCo(s)’	25.04.	29.04.	05.05.	10	01.09.
‘Gala Must®’	25.04.	05.05.	07.05.	12	02.09.
‘Galaxy’	27.04.	02.05.	08.05.	11	03.09.

In average, the earliest date of beginning of flowering was in the clone 'Mondial Gala®Mitchgla' (24.04.), while the latest date of beginning of flowering was in the clone 'Galaxy' (27.04.). The differences in flowering between the years of studies were also noticed. Namely, in 2011, an earlier flowering period was noticed, by 1 – 2 days in comparison with 2012.

Date of full of flowering of examined clones was five to ten days after the beginning of flowering and lasted from 29.04. to 05.05.

The earliest date of the end of flowering was the in clone ‘Gala Schniga®SchniCo(s)’ (05.05.), while the latest date of the end of flowering was the in the clone ‘Galaxy’ (08.05.). The average duration of flowering was 11.5 days (with variation of 10 – 13 days).

Comparing our results with the results of *Lukić et al.* (2011), time of flowering of clones 'GalaMust®' and 'Galaxy' occurred much later in agro – environmental conditions of Sarajevo. The flowering has occurred relatively later in environmental conditions of Sarajevo because of climatic conditions and later start of growing season, but the process and duration of flowering was in accordance with the characteristics of the examined clones.

Time of maturation depends, primarily, on genetical characteristics and environmental conditions, that may influence earlier or later ripening. The time of maturation of the studied clones was from 01.09. (‘Gala Schniga®SchniCo(s)’) to 03.09. (‘Galaxy’). Comparing the years of studies, it can be concluded that the differences in the time of maturation for the same clones were not big.

The average time of maturation of clones 'GalaMust®' and 'Galaxy' was 26.08.) and 25.08. respectively in the region of Cacak (*Lukić et al.*, 2011). Earlier time of maturation for the same clones has been registered in the region of Topola (*Milatović et al.*, 2009), where the clone 'Galaxy' matured on the 20<sup>th</sup> of August and the clone 'GalaMust®' on the 21<sup>st</sup> of August. According to *Atay et al* (2010) and *Bozbuğa and Pırlak* (2012), clone 'Galaxy' had earlier time of flowering and maturing.

Cultivar 'Gala' and its clones according to the time of maturing, belong to the group of autumn apple cultivars.

According to *Akhtar et al.* (2002), clones of apple 'Gala' ripening in mid-August, or 135 days from the beginning of flowering.

The differences between our results and results of other authors can be explained by the different climate conditions.

*Physical characteristics of the fruit.* Physical characteristics of examined clones of 'Gala' were studied immediately after harvesting. The results of physical characteristics of the fruit of 'Gala' apple clones are presented in Table 2.

Table 2. Fruit properties of 'Gala' apple clones in conditions of Sarajevo (average, 2011-2012)

Cultivar	Fruit weight (g)	Fruit length (cm)	Fruit width(cm)	Shape factor (L/W)
'MondialGala®Mitchgla'	168.36 b	6.79	7.58	0.89
'Gala Schniga®SchniCo(s)'	164.10 bc	6.49	7.25	0.89
'Gala Must®'	163.32 c	6.83	7.74	0.88
'Galaxy'	175.38 a	6.60	7.29	0.90

Means followed by the same letter do not differ significantly according to Duncan's multiple range test at  $P=0.05$

The weight of fruit is one of the most important pomological characteristics because it affect a number of other properties, primarily yield, which is the ultimate aim of any production. In our study, the weight of fruit ranged from 163.32 g in clone 'Gala Must®' to 175.38 g in clone 'Galaxy'. Clone 'Galaxy' had statistically significant highest fruit weight than other clones.

Clone 'GalaMust®' had statistically significant lowest fruit weight than other clones in 2011 year, but same clon had statistically significant highest fruit weight than other clones in 2012. year.

According to *Luki et al.* (2011) clone 'GalaMust®' had highest fruit weight, and clon 'Galaxy' had lowest fruit weight in the region of Cacak.

Lower fruit weight of clones 'GalaMust®' and 'Galaxy' was found in study of *Milatovi et al.* (2009) in comparison to the results of our study. Comparing our results to the results of *Miloševi et al.* (2007), and *Atay et al.* (2010) we can see that fruit weight of clone 'Galaxy' is higher in our research. Depending on the year of study, clone 'GalaMust®' had lower or higher fruit weight (*Krzysztof et al.*, 2005). Higher fruit weight of clone 'Galaxy' was found in our study in comparasion to the results of *Bozbu a and Pirlak* (2012). According to the literature, fruit weight of examined clones may be a limiting factor in the growth of these clones, but in our study clones had acceptable values of fruit weight. The average length of fruit ranged from 6.49 to 6.83 cm, while the width of fruit was 7.25 to 7.74 cm.

Highest length and width of fruit was found in clone 'GalaMust®', while the lowest length and width was found in clone 'GalaSchniga®SchniCo(s)'. On the basis of fruit dimensions, fruit shape factor was calculated. That value was the lowest in clone 'Gala Must®' (0.88), and the highest in clone 'Galaxy' (0,90).

The results of yiel of the fruit of 'Gala' apple clones are presented in Table 3.

Table 3. Yields of ‘Gala’ apple clones in conditions of Sarajevo (kg/ha and kg/tree)

Cultivar	Yield of ‘Gala’ apple clones					
	2011.		2012.		Average	
	kg/ha	kg/tree	kg/ha	kg/tree	kg/ha	kg/tree
‘MondialGala@Mitchgla’	30,088.80	9.93	16,755.90	5.53	23,422.35	7.73
‘Gala Schniga@SchniCo(s)’	34,945.89	11.53	21,229.99	7.00	28,087.94	9,265
‘Gala Must@’	26,048.91	8.59	18,786.00	6.20	22,417.455	7,395
‘Galaxy’	1,896.78	0.62	15,144.84	4.99	8,520,81	2,805

Comparing yield between the years, the results showed that the highest yield was in 2011 year and only in clone ‘Galaxy’ the highest yield was in 2012. The lowest yield per tree and per hectare had clone ‘Galaxy’ (2.805 kg/tree, or 8,520.81 kg/ha), and the highest yield per tree and per hectare had clone ‘Gala Schniga@SchniCo(s)’ (9.265 kg/tree, or 28,087.94 kg/ha ).

*Milatovi et al.* (2007) and *Luki et al.* (2011), reported higher yield for clones ‘Galaxy’ and ‘Gala Must@’, while *MiloseviC et al.* (2007) reported value of yield for the clone 'Galaxy' of 23.076 kg/tree. Higher yield of the clone 'Galaxy' has also obtained in studies of *Atay et al.* (2010) and *Bozbu a and Pirlak* (2012).

Lower yields of the tested clones were expected, because the testing was done at a relatively young orchard .

### Conclusion

On the basis of two-year investigations of the phenological characteristics, physical characteristics of the fruit and yield of ‘Gala’ apple clones in the region of Sarajevo we have made the following conclusions:

Flowering of studied cultivars started in the third decade of April, and it lasted 13.6 days (on average).

Average time of maturation was from 01.09. to 03.09.

The weight of fruit ranged from 163.32 g to 175.38 g.

Clone ‘Galaxy’ had significantly highest fruit weight than other clones.

Highest lenght and width of fruit was in clone 'GalaMust@', while the lowest lenght and width was in clone 'GalaSchniga@SchniCo(s)'.

Fruit shape factor ranged from 0.88 to 0.90.

The lowest yield had clone ‘Galaxy’ (2.80 kg/tree, or 8,520,81 kg/ha), and the highest yield had clone ‘Gala Schniga@SchniCo(s)’ (9.26 kg/tree, 28,087.94 kg/ha).

Taking all into account,, all of four clones of apple ‘Gala’ (‘Mondial Gala@ Mitchgla’, ‘Gala Schniga@ SchniCo(s)’, ‘Gala Must@’, ‘Galaxy’) can be recommended for the advancement of Bosnia and Herzegovina apple assortment.

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**FIRST RESULTS OF INDUSTRIAL PROPAGATION OF WALNUT (*J. REGIA* L.) IN BULGARIA BY THE HOT CALLUS METHOD, USING HOT WATER INSTALLATION**

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**Abstract**

The experiment was carried out at the Fruit-Growing Institute – Plovdiv, Bulgaria. The principles of the hot callus method of walnut propagation were applied. A hot water installation was built for maintaining a temperature of 27°C ( $\pm$  1°C) at the place of grafting. The installation comprised of a boiler, a pump, metal tunnels with doors, soil heating pipes, valves, etc.

The principles of the installation and the elements of the technological process were described. The results showed that successfully propagated walnut plants were obtained by applying the hot water installation. The percentage of the successfully propagated plants was 70.0% when cool-stored scions were used and 82.0% when using unstored scions. It was concluded that the hot water installation could be applied in practice.

**Key words:** walnut(*J. regia* L.), propagation, hot callus, hot water installation.

**Introduction**

Propagation of walnut(*J. regia* L.) is more difficult compared to most of the fruit species. It is due to the low rate of callus formation and the presence of high concentration of phenolic compounds (Rongting & Pinghai,1993). Because of walnut heterozygosity, propagation by seeds does not lead to inheritance of the characteristics of a certain variety. For that reason, different methods of walnut propagation have been investigated all around the world. Propagation with cuttings is difficult (Gautam,1990) and at present budding and grafting are the most popular inoculation techniques in the production of walnut trees (Gandev, 2007).

Patch budding is most commonly used in walnut propagation. This is one of the oldest and popular techniques for propagation in a nursery in the open (Kuniyuki & Forde, 1985). Scientific literature reported different data on the efficiency of this inoculation method (Dzhuvinov et al., 2010).

In the recent years, local heating of the graft union with a hot callusing pipe was successfully applied (Lagerstedt,1981; Avanzato, 1997; Avanzato & Tamponi, 1988; Gandev, 2009). The trial methods for local heating of the graft union have been different in the different experiments but the purpose is the same – to achieve a temperature of 26-27°C. According to Lagerstedt (1979), Millikan (1984) and Wilbur et al. (1998), the optimal temperature for walnut callus formation is 26-27°C.

The aim of the present investigation was to create, test and evaluate the hot water installation for walnut propagation under production condition.

## Materials and methods

### Principles of the hot water installation for grafted walnut trees

The experiment for industrial propagation of walnut (*J. regia* L.) by the hot callus method was carried out in 2013 at the Fruit Growing Institute – Plovdiv. A hot water installation for grafted plants was built indoors and tested for its efficiency. The installation is a close water system, in which the circulating hot water maintains a permanent temperature in the heated tunnels. The distance between the separate tunnels is 1.2 m. The elements of the installation comprise of a boiler, a pump, metal tunnels with doors, soil heating pipes placed in the tunnels on perlite, valves and fasteners.

Water in the boilers was maintained at 50°C ( $\pm$  1°C). The temperature in each tunnel depended on the quantity of the water flowing through the soil heating pipe placed at the bottom of the tunnel. Water flow in the tunnels was controlled by valves.

### Walnut Grafting

520 walnut plants of ‘Izvor 10’ cultivar were grafted in the period 11-13 March. Two types of scions were used in both variants of the trial:

First Variant – scions stored for three months in a refrigerator at a temperature of 2-5°C and

Second Variant – scions collected and grafted on the same day.

Cleft grafting was carried out. During the grafting procedure, the vascular cambium of the scion and the rootstock were adhered to each other. The graft was fixed with a rubber cord, which was not tight, in order to allow the exit of the surplus moisture at the place of grafting. According to Rongting & Pinghai (1993) excess bleeding caused the formation of anaerobic conditions at the graft juncture, resulting in a decrease of the graft survival rate. One year old seedlings of *Juglans regia* L. were used as rootstocks. Scions were kept in water for one day to increase the moisture content before grafting. The root tip of each rootstock was cut in order to stimulate the formation of new lateral rootlets. The scion tops were immersed in warm paraffin to prevent water loss (Fig. 1)



Fig. 1. Grafted plants before placing for callus formation.

The grafted plants were laid horizontally over the tunnels, transversely to the longitudinal axis of the tunnel, the place of grafting being exactly at the heated point. The roots of the plants,



which were outside the tunnel, were covered with wet sand. The tunnel doors were tightly closed and covered with plastic folio to decrease moisture evaporation (Fig. 2).



Fig.2 .Heated tunnels stocked with grafted plants.

#### *Caring for the grafted plants*

Heating was provided for the grafted plants for four weeks, the temperature of  $27^{\circ}\text{C}$  ( $\pm 1^{\circ}\text{C}$ ) being maintained at the place of grafting. The air temperature in the premise where the installation was built, was maintained within  $10$  to  $18^{\circ}\text{C}$  by a convector. Plant roots were periodically wetted every few days. When necessary, the perlite in the tunnel was also wetted to provide uniform moisture levels at the place of grafting. In cases of rootstock suckering, the suckers were immediately removed. At the end of the fourth week, when the process of callus formation finished, the heating installation was switched off.

Successfully grafted plants (Fig. 3) were potted and adapted for a month (Fig. 4). At the end of the period of adaptation, the percentage of the successfully propagated trees was reported.



Fig. 3.Plants after callus formation.



Fig. 4. Potted plants.

The experimental design was a complete randomization with five replications per treatment and 20 grafts in each replication.

The data obtained were statistically processed by Duncan's test (Steele & Torrie, 1980).

#### Results and discussion

Obtained results showed that the use of the constructed hot water installation contributed to the success of walnut propagation (Tabl. 1). Throughout the heating period the reported temperature in the tunnels was 27°C ( $\pm 1^\circ\text{C}$ ), which showed the reliability of the installation and as an important precondition for successful callus formation in the grafted plants (PaunoviC et al., 2010).

**Tabl. 1.** Percentage of successfully propagated plants

<b>Variants</b>	<b>Percentage of successfully propagated plants</b>
1. Unstored scions	82.0 a
2. Cool-stored scions	70.0 b

Statistically different at P=5%

Under the climatic conditions of Bulgaria, the buds of the mother walnut trees suffer severe frost bites quite often (Gandev, 2012), which sometimes compromises winter grafting of walnut. The results obtained in the carried out experiment showed that in such years the scions collected before the winter frosts, could guarantee a good percentage of successful grafting. Data presented in Tabl. 1 showed that when using scions collected immediately before grafting, the graft survival rate was 82.0% (Var. 1), while when using stored scions (Var.2), it was lower – 70.0%, the difference being statistically significant.

In the process of the study a certain cultivar-specific variation was established concerning the graft survival percentage, which could be explained by the varietal differences. Concrete data were not presented at that stage of the study. It was only established that among the three

grafted cultivars, i.e. ‘Izvor 10’, ‘Sheynovo’ and ‘Silistrenski’, the highest survival percentage was reported for the first cultivar.

### Conclusion

The first results of the carried out study on industrial walnut propagation by the hot callus method, using a hot water installation for heating the place of grafting, gave the grounds to draw the following conclusions:

The described hot water installation contributes to the production of successfully propagated walnut plants;

Walnut propagation is more efficient when using scions collected on the day of grafting compared to scions collected before and stored in a refrigerator.

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**PREDATORS OF ROSY APPLE APHYD, (*DYSAPHIS PLANTAGINEA*) PASS.,  
(HOMOPTERA, APHIDIDAE) IN BULGARIAN APPLE ORCHARDS**

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**Summary**

The presence of predators associated with Rosy apple aphid, *Dysaphis plantaginea*, (Pass.) (Homoptera: Aphididae) was surveyed in two apple orchards in two different ecological regions in Bulgaria during 2012. In the colonies of *D. plantaginea* in both orchards were found 18 predatory species belonging to respective orders Coleoptera – 5 (Coccinellidae 4, Cantharidae 1), Diptera – 4 (Syrphidae 3, Cecidomyiidae 1), Hemiptera – 4 (Anthocoridae 3, Miridae 1), Neuroptera – 4 (Chrysopidae 4), Dermaptera – 1 (Forficulidae 1). Some of the most frequent predators in this research were *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae), *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae) and *Aphidoletes aphidimyza* (Rond.) (Diptera: Cecidomyiidae). In April, when the colonies of *D. plantaginea* were small in numbers, syrphid eggs and larvae were mostly found and less often ladybird eggs and larvae. Other predators such as *Anthocoris nemoralis* (Fabr.), *Orius minutus* (L.) and *Orius majusculus* (Rt.) (Hemiptera: Anthocoridae), *Deraeocoris ruber* (L.) (Hemiptera: Miridae), *Chrysopa carnea* (Steph.), *Ch. septempunctata* (Wesm.), *Ch. perla* (L.) and *Ch. prasina* (Burm.) (Neuroptera: Chrysopidae), *Forficula auricularia* (L.) (Dermaptera: Forficulidae) and *Cantharis fusca* (L.) (Coleoptera: Cantharididae) were also observed to feed in the colonies of *D. plantaginea*. Although these predators are considered to play an important role in the regulation of aphid populations, they did not prevent *D. plantaginea* damage that affected up to 45 % of the terminals in one orchard and up to 43 % in another. Some possible causes for this ineffectiveness are discussed.

**Key words:** Bulgaria, Rosy apple aphid, aphidophagous, biological control

**Introduction**

*Dysaphis plantaginea*, (Pass.) (Homoptera: Aphididae) is one of the most frequent and deleterious aphid species on apple trees in Bulgaria. It is a migratory species with main host *Malus* spp. and intermediate host *Plantago* spp. In a period of mass multiplication the aphids settle on the fruit clusters where they cause rolling up of leaves and deformation of shoots. When feeding they exude toxins which block the growth of fruit which stay small and underdeveloped. In order to prevent from this damage, every year in early spring one to three insecticide treatments are performed. On one hand, these treatments are serious threat to their natural enemies and pollinators. On the other hand they pose a hazard of resistance emergence due to the repeated use of one and the same chemicals. This makes it necessary to develop alternative approaches to control the pests. The growth of resistant cultivars is probably one of the ways to solve the problem. In connection with this, in our country as well as abroad, hybridization programs are developed, including varieties, resistant to particular species of

aphids (e.g. 'Florina' cultivar to the species *D. plantaginea*) (Dapena and Miñarro, 2001, Kutinkova and Djuvinov, 2004).

The use of natural enemies of aphids like bio-agents and leaving strips of wild plant species as suitable habitat for aphidophagous predators (Wyss, 1995; Wyss *et al.*, 1995), or further colonization of local natural enemies (Wyss *et al.*, 1999a), could be other alternative approaches to control of *D. plantaginea*.

In Bulgaria, purposeful research connected with specifying the complex of *D. plantaginea*'s predators has not been carried out until now. The purpose of this research is to study the predatory insect species to *D. plantaginea* and to estimate their effectiveness in the control of its populations.

### Materials and methods

The experiments were carried out in 2012 in two apple orchards in two different ecological regions in Bulgaria. One of orchards is experimental, located on the territory of the Fruit Growing Institute in the city of Plovdiv and the other one - in an abandoned, located on the periphery of the town of Shumen.

The experimental orchard of 0.4 ha in Plovdiv, consisted of 13-year old apple trees, 14 of them resistant and one susceptible ('Golden Delicious') to apple scab, grafted on MM – 106. This orchard was conducted following organic guidelines. In summer months the orchard was sprayed with summer oils to control aphids and granulosis virus to control codling moth. Treatments against other apple pests were not needed. Copper and sulfur preparations were used against fungal diseases.

The abandoned orchard of 0.5-ha in Shumen, consisted of 30-year old cv 'Golden Delicious', 'Red Delicious', 'Fuji' and 'Melrose', grafted on MM – 106. Neither insecticides, acaricides nor fungicides were sprayed in this orchard in the last 10 years.

In order to minimize effects, 10 trees of each orchard, only cv. 'Golden Delicious', were sampled for the presence of *D. plantaginea*. Five shoots of each selected tree were randomly selected and marked with colored plastic strips. From mid April to the moment when all *D. plantaginea* had migrated to a secondary host the 50 selected shoots were examined on a weekly basis for the presence of aphid colonies.

Number and types of predators was recorded weekly during the spring by visual examination on 25 shoots randomly selected among those infested with *D. plantaginea* in the two gardens. Eggs and larvae were brought back to the laboratory in order to determine their species. Predators were reared individually in glass containers on *D. plantaginea* or *Aphis pomi* at 23-25°C and 75% humidity. Identifications of predatory species were made using keys of insect species in the adult stage by Dorohova *et al.* (1989).

### Results

#### Aphids

The percentage of growing shoots with colonies of *D. plantaginea* in both orchards was high, reaching 45 % in some moments (fig. 1 and 2). In the experimental orchard the first colonies were observed in the middle of April and the last ones until the end of June, when the aphids migrated to *Plantago* spp., their second host (fig. 1). The peak of the mass multiplication was registered end of May. The sprays with summer oil on 14<sup>th</sup> May; 28<sup>th</sup> May and 11<sup>th</sup> June did not affect significantly the populations of *D. plantaginea* which could be explained with the fact that the efficacy of contact insecticides in the rolled leaves decrease considerably (Hull & Starner, 1983).

In the abandoned orchard the first colonies were observed in the middle of April as well but the aphids remained on the developing shoots until the first ten days of July (fig. 2). In that orchard the peak of multiplication of aphids was registered in the first half of June.

### Predators

In the colonies of *D. plantaginea* in both orchards were found 18 predatory species belonging to orders Coleoptera – 5 (Coccinellidae 4, Cantharidae 1), Diptera – 4 (Syrphidae 3, Cecidomyiidae 1), Hemiptera – 4 (Anthocoridae 3, Miridae 1), Neuroptera – 4 (Chrysopidae 4), Dermaptera – 1 (Forficulidae 1) (fig. 3 and 4). The most frequent and numerous of these were the species of family Syrphidae, Cecidomyiidae and Coccinellidae, while the rest of the predator species were found less often and in lower numbers. The most abundant and the first observed syrphid was *Episyrphus balteatus* (De Geer), although *Scaeva pyrastris* (L.) and *Syrphus ribesii* (L.) were also recorded. The most common and frequent species of coccinellids was *Adalia bipunctata* (L.); they were 85% of the total number of all Predatory ladybirds. In the *D. plantaginea* colonies were observed other coccinellid species like *Coccinella septempunctata* (L.), *Propylea quatuordecimpunctata* (L.) and *Adalia decempunctata* (L.) as well as the cecidomyiid fly – *Aphidoletes aphidimyza* (Rondani).

At the beginning of vegetation in *D. plantaginea* colonies were found mostly predatory ladybirds and syrphid flies. In the experimental orchard, in more than 70% of the colonies were observed eggs and larva's of syrphid flies while the coccinellids in both orchards were lower in number (fig. 3 and 4).

The third most frequent predator in this study, the cecidomyiid fly *A. aphidimyza* was found for the first time in both orchards at the end of the first decade of May. The number of this predator in the following weeks increased fast and at the beginning of June its presence in the colonies of *D. plantaginea* reached 65% (Fig. 3 and 4).

In May in the colonies of *D. plantaginea* larvae of syrphid flies were most often found but in June, with increasing numbers of aphid populations, mostly larvae of coccinellid and cecidomyiidae flies were observed. Along with these predators in the colonies was occurrence of anthocorids and mirids like *Anthocoris nemoralis* (Fabr.), *Orius minutus* (L.) and *Orius majusculus* (Rt.) (Hemiptera: Anthocoridae), *Deraeocoris ruber* (L.) (Hemiptera: Miridae), and chrysopids (*Chrysopa carnea* (Steph.), *Ch. septempunctata* (Wesm.), *Ch. perla* (L.) and *Ch. prasina* (Burm.) (Neuroptera: Chrysopidae), whose presence in the abandoned orchard was stronger than in the experimental one. At the beginning of June in the abandoned orchard these predators could be found in 40% of the colonies of *D. plantaginea*. In that orchard, in addition to these predators in the *D. plantaginea* colonies were found other predatory species like *Forficula auricularia* (L.), (Forficulidae) and *Cantharis fusca* (L.) (Cantharidae) but mostly rarely and in low number.

Spiders were rarely observed in both apple orchards and although they were not seen feeding on *D. plantaginea*, they can play an important role in decreasing aphid populations principally in autumn (Wyss *et al.*, 1995).

In *D. plantaginea* colonies was reported significant presence of ants. These insects develop in symbiosis with aphids and their presence in the colonies of *D. plantaginea* is probably not accidental but with a particular mission to protect aphids from the negative impact of their natural enemies.

### Discussion

In previous studies Wyss *et al.* (1999b) demonstrated the efficacy of *E. balteaus*, *A. bipunctata* and *A. aphidimyza* predators on *D. plantaginea* colonies. In this study these three species are some of the most frequent and common predators too. Although they play an important role in regulating the populations of *D. plantaginea* aphids, they are not capable to reduce *D. plantaginea* populations below under an economic threshold (1-2 infested shoots after flowering) (Fig. 1 and 2).

There might be several main reasons for the inability of these predators to prevent the damage caused by *D. plantaginea*. One of them is the fast reaction of apple leaves as a result of aphids feeding. The onset of symptoms can be seen within 24 hours (Forrest & Dixon, 1975). Although the aphid populations can be controlled by their natural enemies, the typical for the species symptoms of damage could be seen on the leaves and fruit. In this case the significance of aphidophagous could be bigger only if more resistant cultivars are grown. Secondly, there are essential differences in the speed the predator and prey develop. When the predator develops relatively slower than its prey, it is incapable to response adequately to the growing number of prey. Due to this the predator is not as effective as the time for its development is similar to that of the prey. This is the case with predator ladybirds which feed with aphids (Dixon *et al.*, 1997). Thirdly, the aphidophagous coccinellids behave as if they are “prudent predators” (Hemptinne and Dixon, 1998). The theory of optimal feeding of the aphidophagous coccinellids forecasts that in the aphid colony there will be optimal number of eggs whose maximum will be equal to the biomass of the offspring. Theory also predicts that if the egg-laying females lay the optimal number of eggs, their offspring will not affect substantially the size of aphid colonies (Kindlmann and Dixon, 1993). Field and experimental results confirm this prognosis model. Thus gravid females respond to both the abundance and quality of their prey, avoiding aphid colonies that are already exploited and/or too old to support the full development of the ladybird offspring, and so they are not effective bio-agents (Hemptinne *et al.*, 1992; Hemptinne *et al.*, 1993). There is also evidence that aphidophagous syrphids behave similarly (Hemptinne *et al.*, 1993; Scholz and Poehling, 2000).

Parasitism has not been studied in this work, and although some parasited aphids were observed, parasitoids seem to play a minor role in regulating populations of *D. plantaginea*, probably due to the host alternation of this species, ant attendance and hyper parasitism (Cross *et al.*, 1999). Interspecies predation could also be a reason for this, but it was not a subject of this study, although, according to Wyss *et al.* (1999b), in other experiments the two most numerous predators in our apple orchards *A. bipunctata* and *E. balteatus* showed that they not only affect but have a negative effect on aphid populations which is explained by an additional method.

This study clarifies to a certain degree the efficacy of *D. plantaginea* predators and increases the need of similar future research in order to improve the natural control of populations of this aphid species. It could serve as the basis for developing such strategies as artificial breeding and placing of natural or introduced aphid predators or preservation and stimulation of the natural enemies' aphidophagous by leaving zones of wild vegetation.

### **Conclusion**

Although these predators are considered to play an important role in the regulation of aphid populations, they did not prevent *D. plantaginea* damage that affected up to 45 % of the terminals in one orchard and up to 43 % in another.

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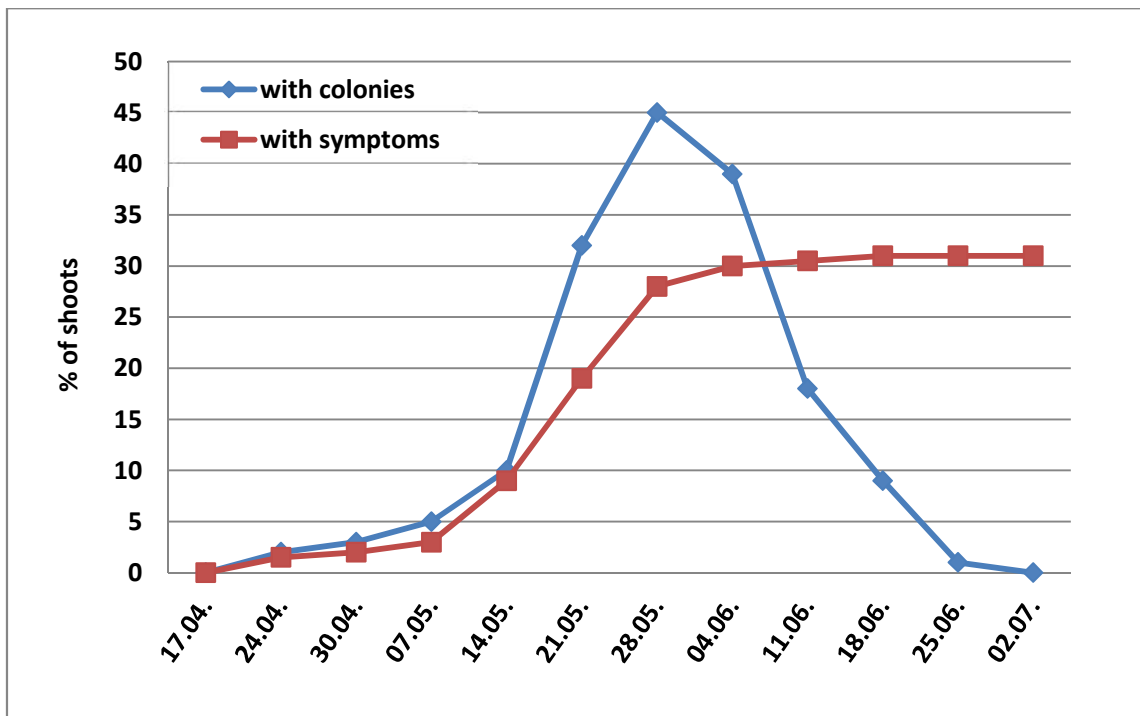


Fig.1. *D. plantaginea* occurrence and shoot damage in the experimental apple orchard in Plovdiv in 2012.

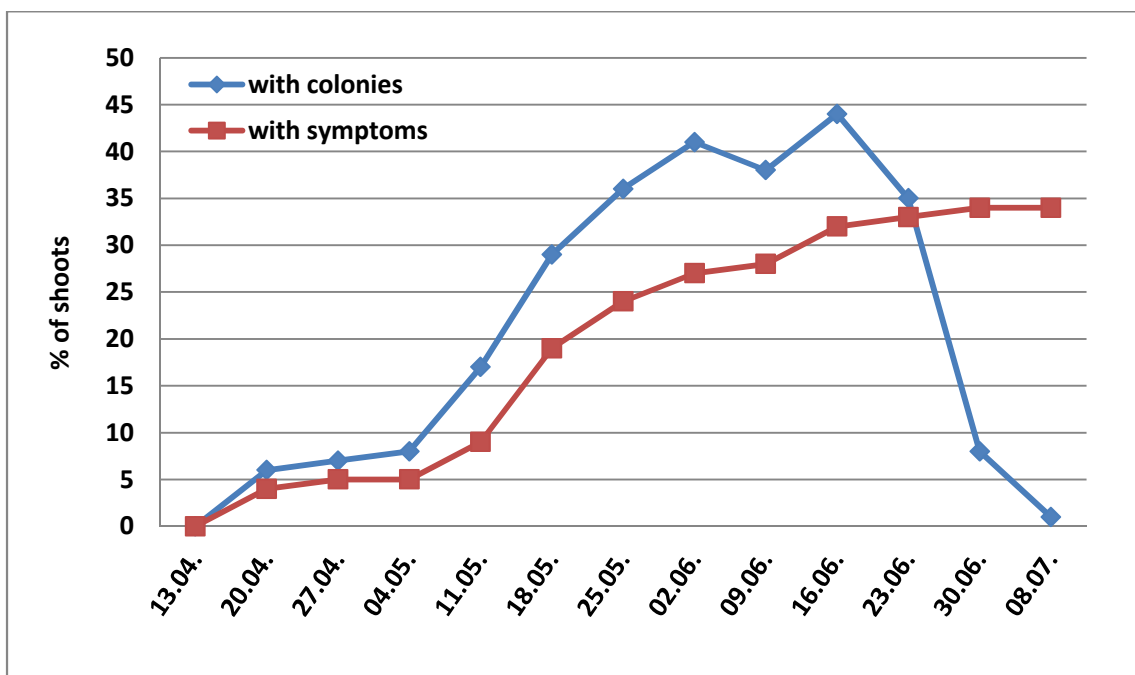


Fig.2. *D. plantaginea* occurrence and shoot damage in the abandoned apple orchard in Shumen in 2012.

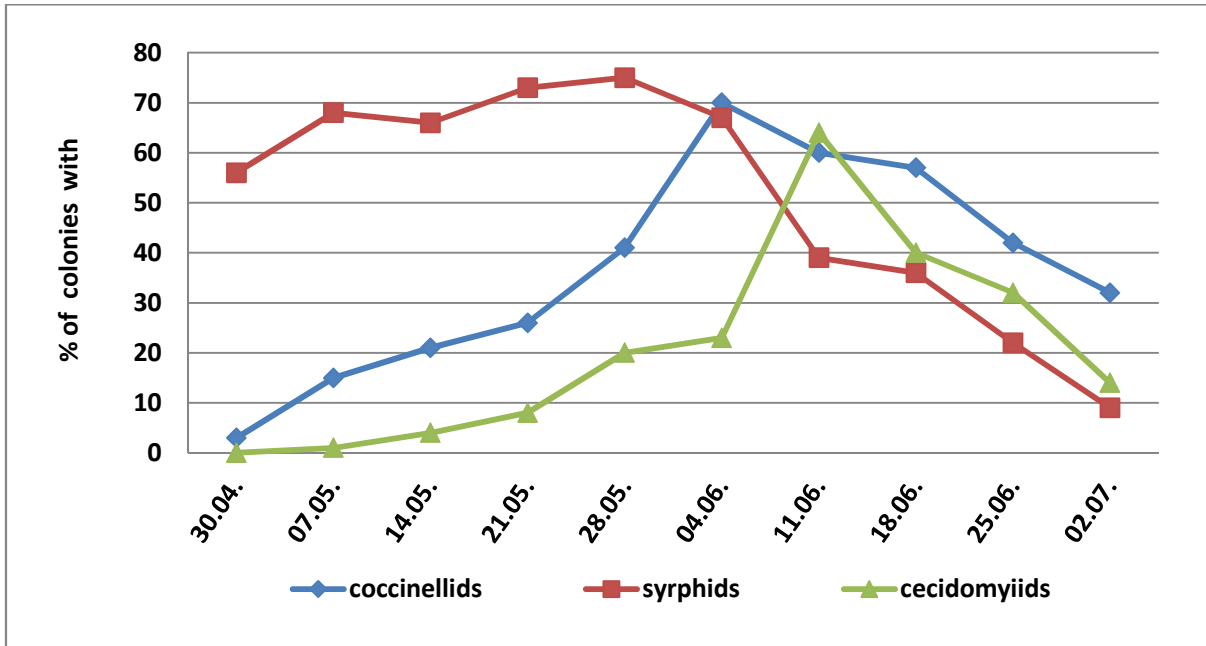


Fig.3. Coccinellid, syrphid and cecidomyiidae occurrence on *D. plantaginea* colonies in the experimental orchard in Plovdiv in 2012.

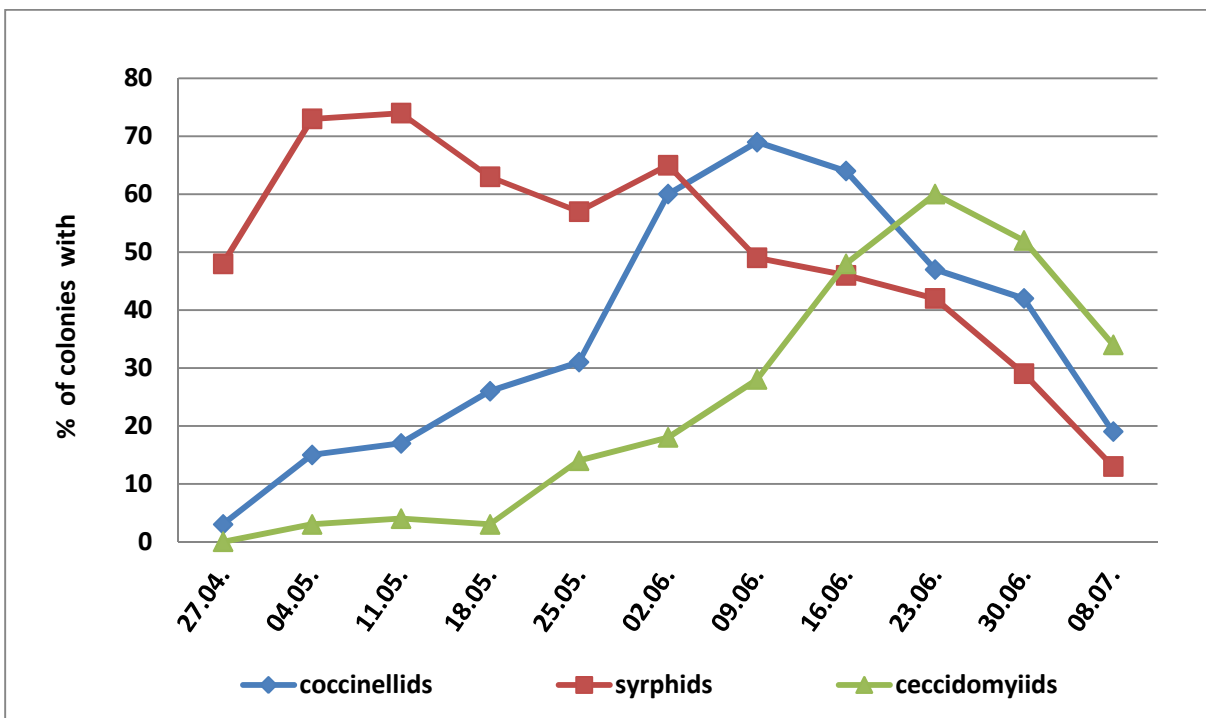


Fig.4. Coccinellid, syrphid and cecidomyiidae occurrence on *D. plantaginea* colonies in the abandoned apple orchard in Shumen in 2012.

**POSTHARVEST EVALUATION OF CUT "WHITE SIM" CARNATION FLOWERS**

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**Abstract**

Two separated experiments were performed in order to evaluate the effects of different concentrations of silver nanoparticles, methanol extract of *Crambe orientalis* L. plant, S-carvone, Salicylic acid, Humic acid, Silver thiosulphate and ethylene on longevity and some quality characteristics of cut 'White Sime' carnation flowers (*Matthiola incana*) based on completely randomized design with 5 replication. These experiments were carried out in postharvest laboratory of Mohaghegh Ardabili University in 2011. The results of first experiment showed that ethylene reduced the vase life of flowers and STS at all concentrations blocked the ethylene effects and increased the longevity of carnation flowers. In second experiment, STS and nano-silver increased flower vase life, while Humic acid, S-carvone and *Crambe orientalis* L. extracts did not influence longevity of cut carnation flowers. However, the higher levels of *Crambe orientalis* L. extract positively influenced the RFW of flowers. The results also showed that flowers subjected to 0.25 and 0.5 mM of STS maintained solution uptake until the last day of experiment and showed higher rate compared to control and other treatments. Moreover, the lower concentration of STS was more efficient than higher ones even in flower subjected with 10 and 100  $\mu\text{l l}^{-1}$  of ethylene.

**Keywords:** Fresh weight, Longevity, Postharvest, Solution uptake.

**Introduction**

Postharvest senescence is a major limitation to the marketing of many species of cut flowers and considerable effort has been devoted to developing postharvest treatments to extend the marketing period (Ichimura *et al.*, 1998., Shimamura and Okabayashi, 1997). Carnation is a typical ethylene sensitive flower and the senescence of this flower (*Dianthus caryophyllus* L.) is accompanied by a marked increase in ethylene synthesis and a concomitant climacteric rise in respiration. The postharvest life of ethylene sensitive flowers can be considerably increased by silver thiosulfate (STS) treatment. For instant, pre-treatment of cut carnation flowers with silver thiosulfate complex (STS) prevents the climacteric rise in ethylene production and delays senescence of flowers (Yangkhamman, *et al.*, 2005; Basiri *et al.*, 2011). Also, silver thiosulphate competes with ethylene for the same site of action and therefore reduces the negative effect of ethylene (Nowak and Rudnicki, 1990). STS is known to suppress autocatalytic ethylene production by inhibition of ethylene action (Ichimura *et al.*, 1998., Shimamura and Okabayashi, 1997).

Recently, finding new compounds for increasing the flower vase life without pollution risks to substitute with STS have been very important.

Salicylic acid has been tested for control of postharvest diseases. It has been shown that salicylic acid in concentrations of 100, 500, and 1000 ml/l-1 was effective in controlling at least four pathogens of orange [*Citrus sinensis* (L.) Osbeck] and potato (*Solanum tuberosum*

L.). Most of the research using salicylic acid is directed to induction of systemic acquired resistance (SAR) in hosts against the attack of pathogens (Capdeville *et al.*, 2003).

The effects of some plant extracts have been evaluated on vase life of cut flowers. For instant, 25% of rosemary extract has extended vase life of cut carnation flowers (*Dianthus caryophyllus* cv. 'white liberty) up to 24.6 days in laboratory conditions (Basiri *et al.*, 2011).

Nanometer sized silver ( $\text{Ag}^+$ ) particles (NS) are considered to more strongly inhibit bacteria and other microorganisms than Ag in various oxidation states;  $\text{Ag}^0$ ,  $\text{Ag}^+$ ,  $\text{Ag}^{2+}$  and  $\text{Ag}^{3+}$  (Furno *et al.*, 2004). Usage of nano-silver compounds (NS) as a pulse and vase solution treatment for cut flowers is relatively new (Liu *et al.*, 2009) and has demonstrated importance as an antibacterial agent (Morones *et al.*, 2005). NS releases  $\text{Ag}^+$  which has been reported to interact with cytoplasmic components and nucleic acids, to inhibit respiratory chain enzymes and to interfere with membrane permeability (Park *et al.*, 2005).

S-carvone is a natural compound with anti-microbial activity which has been used to increase the vase life of some ornamental plants. Previously, the positive effects of s-carvon on vase life, fresh weight and solution uptake of some cut flowers such as *Baeckea frutescens* have been reported (Damunupola *et al.*, 2010).

Humic acid is a natural organic polymer which is produced as a result of degradation of soil organic material such as peat and lignin. In many systems, humic substances behave similarly to cytokine's.

The purpose of this study was to evaluate the physiological effects of different compounds used to formulate preservatives solutions for floral stems conservation and their influence in the postharvest quality of cut carnation flowers.

## Material and methods

### *Plant material*

Cut carnation flowers (*Dianthus caryophyllus* cv. "White Sim") were obtained from a commercial greenhouse (Tehran) at the commercial stage and transported to the postharvest laboratory of Mohaghegh Ardabili University, Ardabil. Two different experiments at two different times were conducted to evaluate the postharvest characteristics of cut carnation flowers.

### *First experiment*

Flowers were pulse treated with different concentrations of silver thiosulfate (0.5, 1 and 2 mM), S-carvon (0.3, 0.6 and 1.2 mM), Salicylic acid (0.5, 1, 1.5 and 2 mM), Humic acid (10, 100, 500 and 1000  $\text{mg/l}^{-1}$ ), Nano-silver (10, 20, 50 and 100  $\text{mg/l}^{-1}$ ), extract of *Crambe orientalis* L. (10, 50, 100 and 500  $\text{mg/l}^{-1}$ ) and ethylene (1, 10 and 100  $\mu\text{l/l}^{-1}$ ) for 24h at 22°C. Deionized water used as a control at the same condition.

### *Second experiment*

*First part:* To confirm the results of first experiment and also to find exact effective concentrations of various compound, flowers were pulse treated with different concentrations of silver thiosulfate (0.25, 0.5, 0.75, 1, 1.5 and 2 mM), S-carvon (0.3, 0.6 and 1.2 mM), Salicylic acid (1, 1.5 and 2 mM), Humic acid (10, 100, 500 and 1000  $\text{mg/l}^{-1}$ ), Nano-silver (10, 20, 50 and 100  $\text{mg/l}^{-1}$ ) and extract of *Crambe orientalis* L. (B) (10, 50, 100, 500 and 750  $\text{mg/l}^{-1}$ ) for 24h at 22°C. Deionized water used as a control at the same condition.

*Second part:* In this experiment cut carnation flowers firstly treated with different concentrations of silver thiosulfate (0.25, 0.5 and 1 mM) for 2 h and then subjected to various concentrations of ethylene (1, 10 and 100  $\mu\text{l/l}^{-1}$ ) for 24 hours.

Postharvest experiments were carried out under vase life evaluation room conditions of  $22\pm 2^{\circ}\text{C}$ , 60-70% relative humidity (RH) and 12 h photoperiod with cool white fluorescent lamps. The harvested flowers were put into vases containing distilled water and  $10\text{ mg l}^{-1}$  chlorine.

#### *Postharvest assessments*

Longevity was recorded as days after treatment. The vase lives of flowers were considered when the edge of petals started to brown and lost their marketable quality. Relative fresh mass for stems was calculated using the formula:  $\% \text{fresh mass} = (W_t/W_{t=0}) \times 100$ ; where  $W_t$  = weight of stems (g) at Day 0, 2, 4, 6, etc. and  $W_{t=0}$  = weight of the same stem (g) at day 0. Vase solution usage was determined using the formula: Solution uptake ( $\text{ml day}^{-1}\text{g}^{-1}$ , fresh weight) =  $(S_{t-1}-S_t)/W_{t=0}$ ; where,  $S_t$  = solution weight (g) at  $t = \text{Day } 1, 2, 3, \text{ etc.}$   $S_{t-1}$  = solution weight (g) on the previous day and  $W_{t=0}$  = fresh weight of the stem (g) at Day 0.

Some abbreviations which were used in this paper entitled such as STS=silver thiosulfate, NS=Nano-silver, B=extract of *Crambe orientalis* L., E=ethylene, SA= Salicylic acid, HA= Humic acid and SC= S-carvone.

#### *Statistical analyses*

Experiment was conducted in completely randomized design with 5 replications. Data were analyzed with SAS Release 9.1 for Windows. Duncan's Multiple Range Test (DMRT;  $P = 0.05$ ) was used for comparison of treatment means.

## **Results**

### *First experiment*

The results of first experiment showed that the ethylene treatment significantly ( $P < 0.05$ ) reduced vase life of flowers compared to control. On the other words, flowers treated with ethylene lasted four times less than control. However, no significant differences were found among various concentrations of ethylene (Fig. 1).

According to the results of second part of first experiment, it is obvious that STS significantly ( $P < 0.05$ ) increased the vase life of cut carnation flowers compared to control and other treatments. There was a two-fold increase in vase life of flowers subjected to 0.25, 0.5, 1 and 1.5 mM of STS (20, 27, 25 and 21 days after initial treatment) in comparison with other treatments and control. Other treatments including different concentrations of S-carvon, Humic acid, N- silver, Salicylic acid and extract of *Brasica spp* did not significantly ( $P < 0.05$ ) influence flower postharvest life (Fig 2).

### *Second experiment (Part 1):*

The results of these experiments revealed that different treatment significantly ( $P < 0.05$ ) affected vase life, fresh weight and solution uptake.

#### *Vase life*

Results in the first part of second experiment revealed that ethylene could promote the process of senescence and consequently reduced vase life of cut flowers. STS pre-treatment gave a significant extension of flower vase life at least 7.6 days compared to control. However, the STS pre-treatment at highest concentration (1 mM) provided 24.6 days of vase life when flowers were challenged with  $1\ \mu\text{l l}^{-1}$  ethylene, but vase life of untreated flowers (control) was

10 days. The undesirable effects of ethylene were alleviated by application of all four concentrations of STS. It obviously indicates that STS can block ethylene action regardless of its initial concentration. Also, results showed that higher concentrations of STS (0.5 and 1mM) were more effective than lower concentration (0.25 mM) in extending the longevity of flowers (Fig 3).

#### *Relative fresh weight*

It can be clearly seen that relative fresh weight (FW) of cut flower in all treatments increased gradually after the days 7 of the experiment. In contrast, there was a sharp fall in fresh weight of control flowers after 7 days and the downward trend continued to a low of 58% of initial fresh weight at the end of experiment. Despite flowers had been subjected to ethylene, flowers pretreated with different concentrations of STS followed by different concentrations of ethylene maintained FW until the last day (Fig. 4). Plants subjected to 10 and 100  $\mu$ l/l ethylene showed similar results (Fig 5).

#### *Solution uptake*

According to data from Fig 6, it is clearly obvious that, all concentrations of STS alleviated the negative effects of high ethylene rates. However, the rate of solution uptake in STS treated flowers were significantly ( $P < 0.05$ ) higher than control in last day.

Flowers subjected to 0.25 and 0.5 mM of STS continued solution uptake until the last day of experiment and showed higher rate compared to control and other treatments. Moreover, the lower concentration of STS was more efficient than higher ones even in flower subjected with 10 and 100  $\mu$ l/l<sup>-1</sup> of ethylene (Fig 6).

#### *Second experiment (Part 2):*

##### *Vase life*

According to the Fig 7, only STS could significantly ( $P < 0.05$ ) increased vase life of cut carnation flower. Also, Nano-silver (NS) at rates of 20 and 50 mM and S-carvon at 1.2 slightly showed positive effects on flower vase life. There was a dramatic increase in flowers vase life in STS-treated flowers except the highest concentration (2 mM). On the other hands, lower concentrations of STS (0.25 and 0.5 mM) increased flower vase life more efficient than higher concentrations (1.5 and 2 mM). Hence, STS at rates of 0.25 and 0.5 mM increased the vase life of flowers by 15 days, while it did not noticeable at rates 1.5 and 2 mM. Humic acid, S-carvon (except at rate 1.2 mM) and *Brasica Spp* extract din not positively influence longevity of cut carnation flowers. However, Salicylic acid at rate 1.5 mM significantly ( $P < 0.05$ ) decreased flower vase life compared to control (Fig. 7).

##### *Relative fresh weight*

The results of experiment revealed that, lower concentration (10 ppm) of humic acid positively influenced flower fresh weight throughout experiment and had more FW than control. Higher concentration of S-carvone (1.2 mM) was the most efficient treatment and increased fresh weight of cut carnation flowers (Fig. 8).

*Crambe orientalis* L. extract (B) at rates of 100, 500 and 750 ppm, maintained RFW of cut carnation flowers. Whereas, at lower concentrations (10 and 50 ppm) did not influence RFW (Fig. 9). It's concluded that the highest concentrations of NS (100 ppm) and SA (2 mM) had negative effects on RFW of flowers (Fig. 10). In contrast, lower concentrations of NS and SA showed slightly positive impact on RFW of cut carnation flower compared to control. It is clearly obvious that STS at all concentrations had positive effects on RFW, and flowers treated with 0.25, 0.5, 0.75 and 1 mM of STS maintained their initial FW until the last day of experiment (Fig. 11).

### Discussion

Results in this experiments showed that STS plays a key role in the maintenance of the vase life of cut carnation flowers. The STS mechanism in extending the vase life of cut flowers is related to suppression in the induction of autocatalytic production of ethylene, known as primary cause of early senescence of carnation flowers (Ichimura and Hiraya, 1999; Mor et al., 1984; Sexton et al., 1995).

STS pre-treatment had greater efficacy against ethylene treatment. Reid *et al.* (1989) reported that the effect of exogenous ethylene ( $0.5 \mu\text{l l}^{-1}$ ) on rose flowers could be overcome by pre-treatment with STS at a rate of  $0.5 \mu\text{mol stem}^{-1}$ . Elgar *et al.* (2003) reported that exposure of *Leucocoryn ecoquimbensis* inflorescences to  $8 \mu\text{l l}^{-1}$  ethylene for 24 h reduced its vase life from 10 to 5 days and pre-treatment with 1mM STS for 2 h protected flowers, giving a vase life of 9.1 days. Similarly, Macnish *et al.* (2000) reported that STS treatment was effective in providing waxflower with long term protection against ethylene. Newman *et al.* (1998) reported that STS treatment was effective in affording long-term protection of developing buds on cut *Gypsophila paniculata* inflorescences against ethylene. They proposed that the STS complex remained available in the inflorescence and capable of binding to receptors formed as the buds opened into flowers. Delay in fresh weight loss by STS pretreatment in ethylene-treated flowers has also been reported for *Verticordia nitens* (Joyce and Poole, 1993).

The positive effect of NS on vase life and water uptake can be attributed to anti-bacterial effect of this compound. Furno et al. (2004) showed that because of their high surface area to volume ratio, nanometer sized silver ( $\text{Ag}^+$ ) particles (NS) are considered to more strongly inhibit bacteria and other microorganisms than Ag in various oxidation states;  $\text{Ag}^0$ ,  $\text{Ag}^+$ ,  $\text{Ag}^{2+}$ ,  $\text{Ag}^{3+}$ . Also Van Meeteren et al. (2001) reported that  $\text{AgNO}_3$  added to deionised water had a positive effect on *Bouvardia* water status and that use of tap water (containing a mixture of ions) had a similar effect to  $\text{AgNO}_3$  solution. Ions in water, particularly Cations, can enhance flow through xylem vessels (Van Ieperen *et al.*, 2000).

Vase life of cut carnation flower was shortened at 100 ppm of NS. High concentrations of NS may be toxic to cut carnation. The negative effects of higher concentration of NS have been reported in gerbera cut flowers (Liu *et al.*, 2009).

In this experiment the higher levels of *Crambe orientalis* L. extract positively influenced the RFW of flowers. The effect of some plant extracts on extending the longevity of cut flowers has been reported previously. For instant, rosemary's extract increased vase life of cut carnation flowers. This increase on vase life of cut flowers, petals and leaves of cut carnation is caused mainly by decreasing of bacteria concentration in vase solutions.

Salicylic acid did not influence the vase life of cut carnation flowers. This result was in agreement with those of Capdeville *et al.* (2003) who reported that this compound could not increase the longevity of rose flowers.

Overall, sensitivity to ethylene is key issues in the postharvest longevity of cut “White Sim” carnation flowers. The STS mechanism in extending the vase life of cut flowers is related to suppression in the induction of autocatalytic production of ethylene. This difference may be attributable to a residual ‘pool’ of STS in pre-treated flowers that yields silver ions to block newly forming ethylene binding sites. The positive effect of NS on vase life and water uptake can be attributed to its anti-bacterial effect. The higher levels of *Crambe orientalis* L. extract positively influenced the RFW of flowers. This increase on vase life of cut flowers, petals and leaves of cut carnation is possibly caused by decreasing of bacteria concentration in vase solutions. Salicylic acid and Humic acid did not influence the vase life of cut carnation flowers. It seems these compound could not influence ethylene production and may be did not affect the RFW and Solution uptake of cut “White Sim” carnation flowers.

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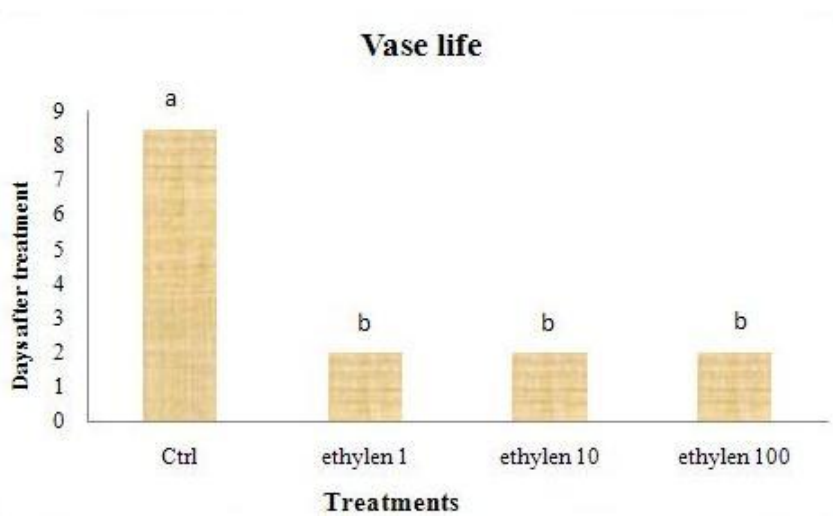
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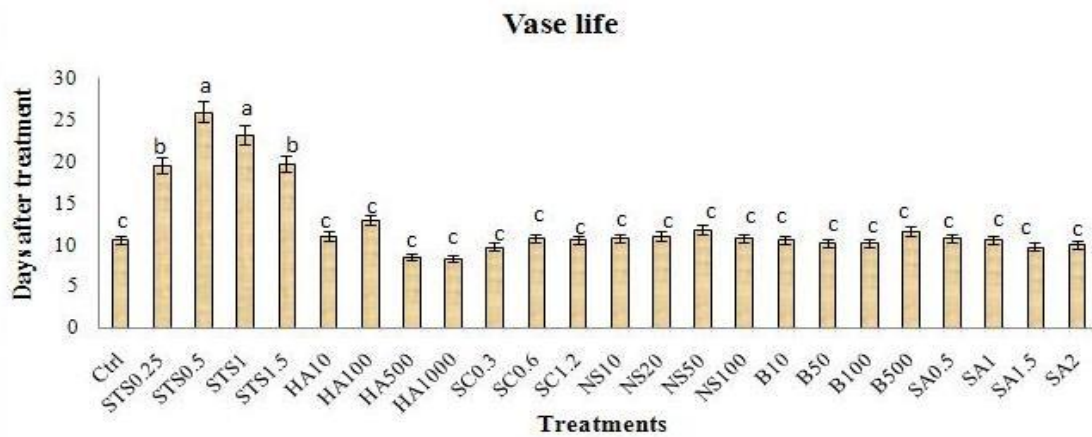
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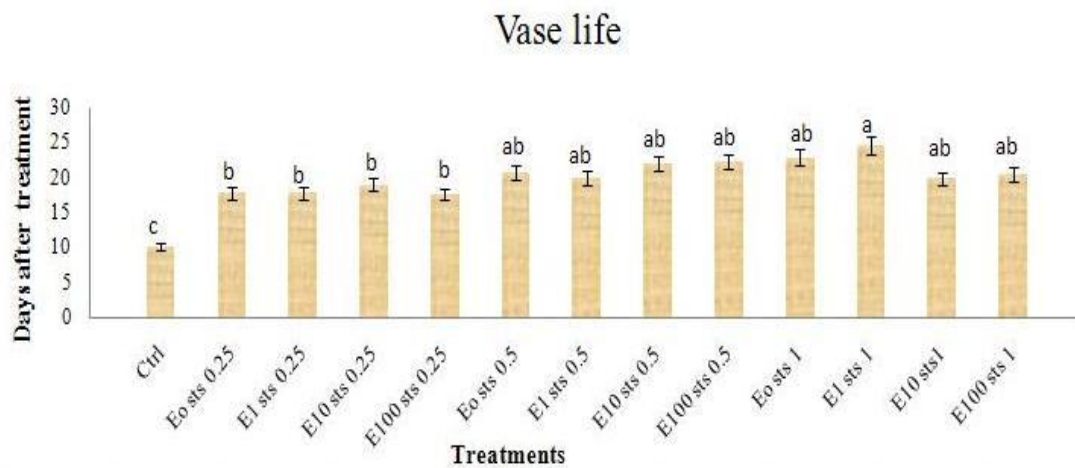
**Figures:**



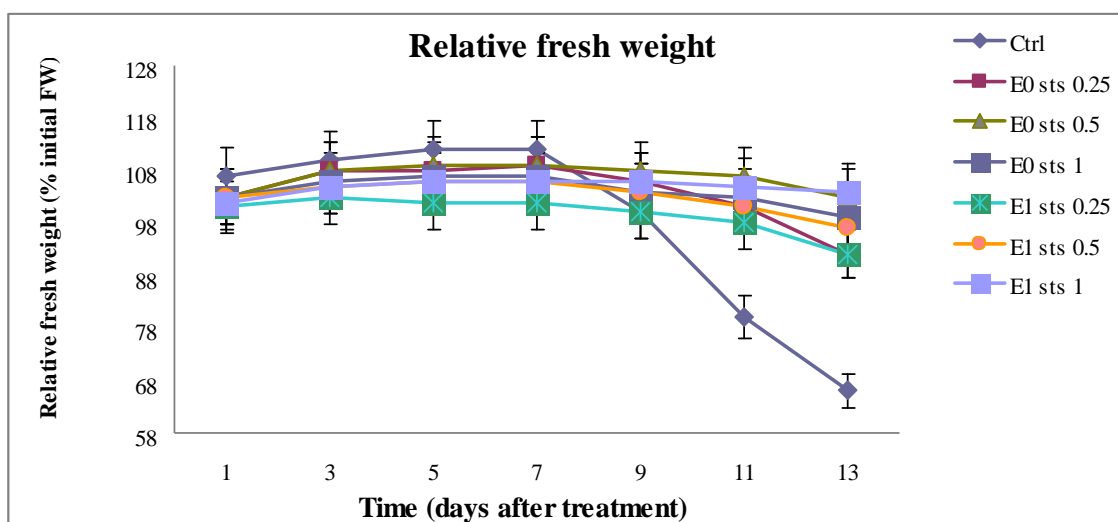
**Fig.1.** The effects of different concentrations of ethylene ( $\mu\text{l}^{-1}$ ) on vase life of flowers.



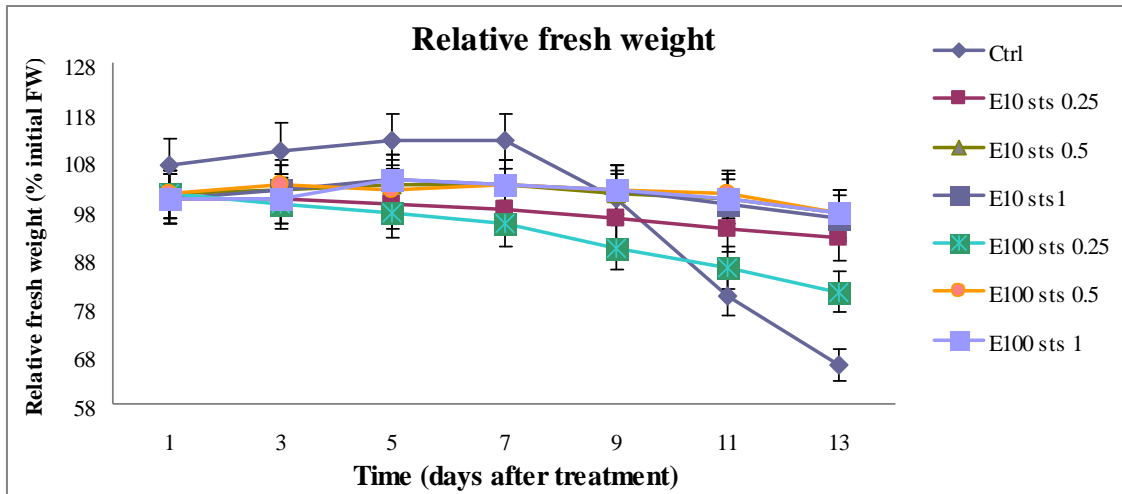
**Fig. 2.** The effects of different treatments on vase life of cut carnation flowers.



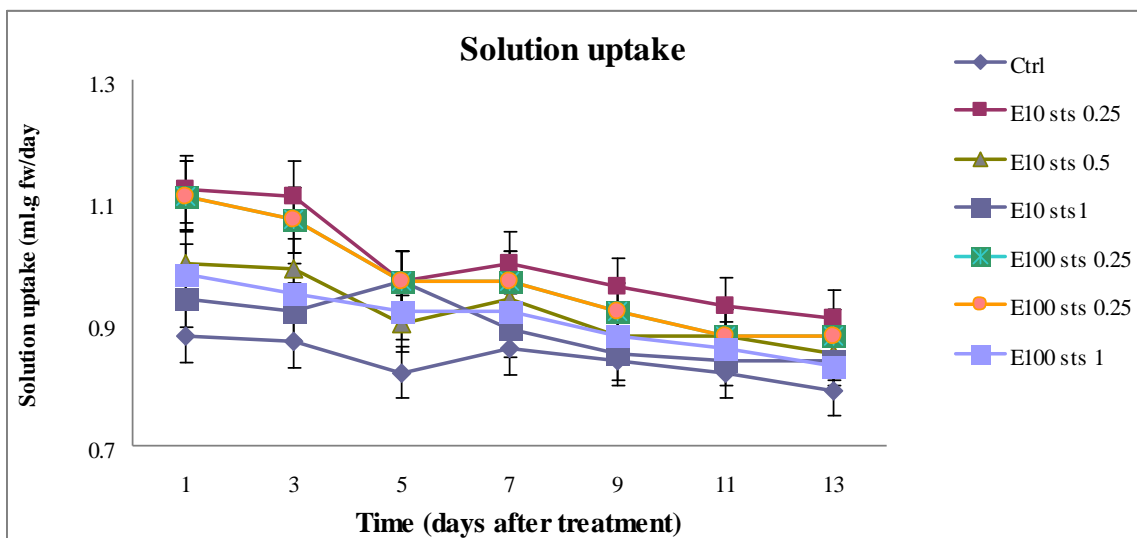
**Fig.3.** The effects of different rates of ethylene and STS on vase life of cut carnation flowers.



**Fig.4.** Effects of ethylene (0 and 1  $\mu\text{l/l}^{-1}$ ) and STS pretreatments (0.25, 0.5 and 1 mM) on relative fresh weight of cut carnation flowers.



**Fig. 5.** The effects of ethylene (10 and 100  $\mu\text{l/l}^{-1}$ ) and STS pretreatments (0.25, 0.5 and 1 mM) on relative fresh weight of cut carnation flowers.



**Fig. 6.** The effects of ethylene (10 and 100  $\mu\text{l/l}^{-1}$ ) and STS pretreatments (0.25, 0.5 and 1 mM) on solution uptake of cut carnation flowers.

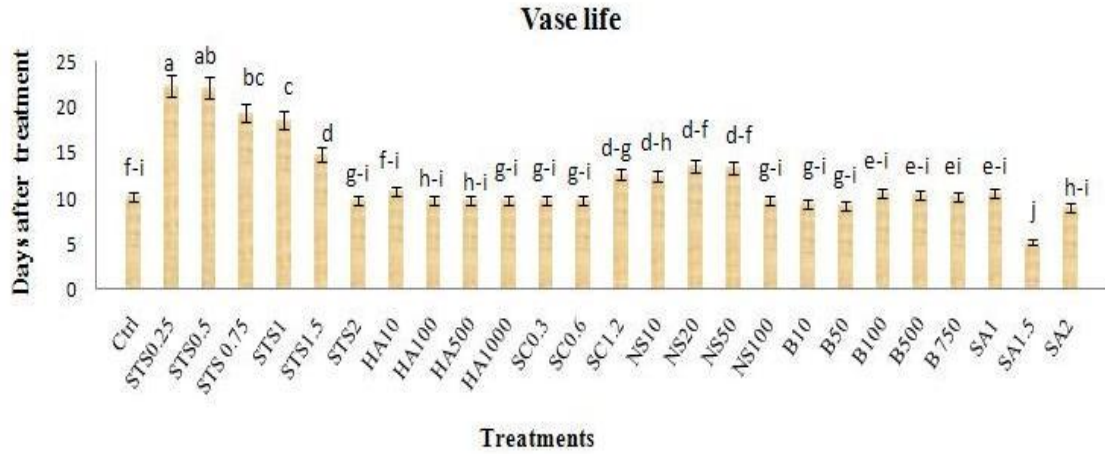


Fig.7. The effects of various treatments on vase life of cut carnation flowers.

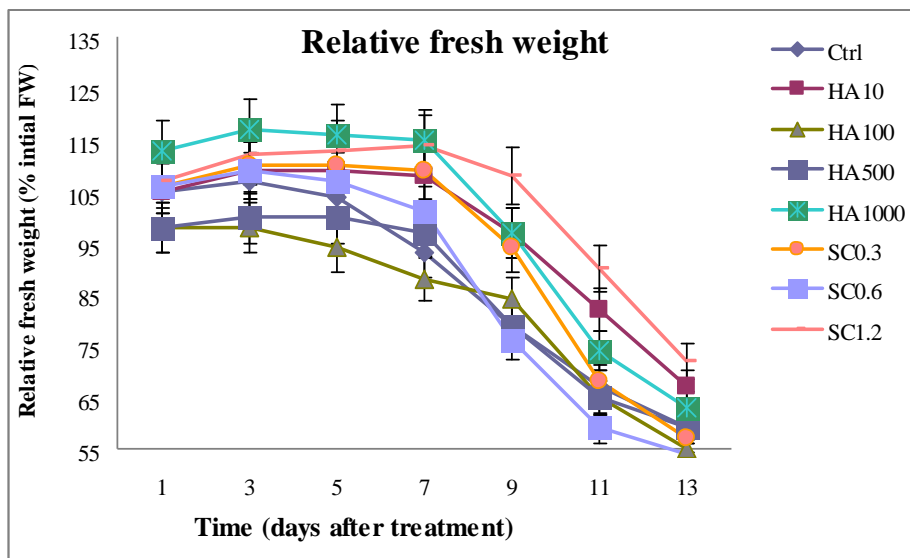
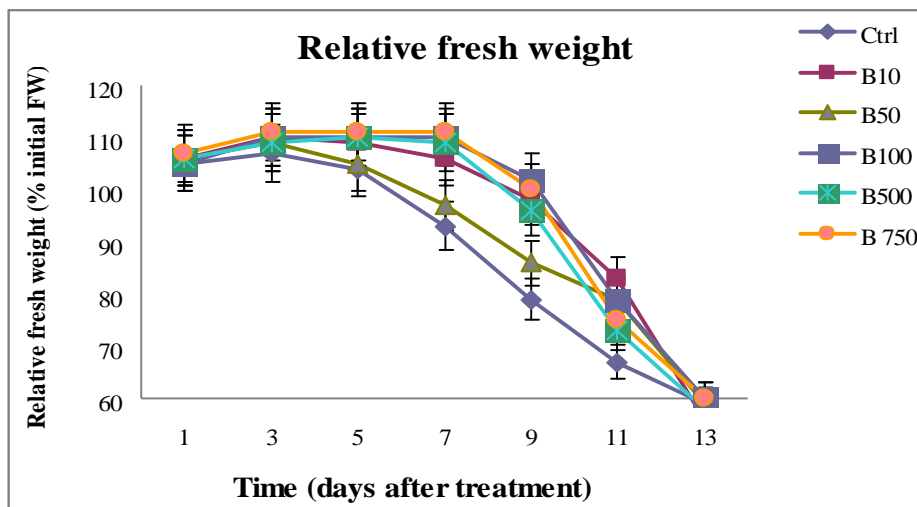
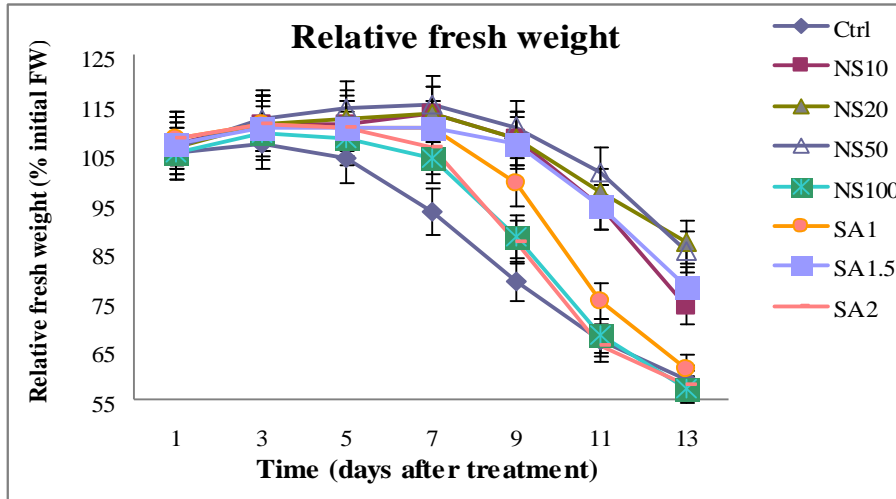


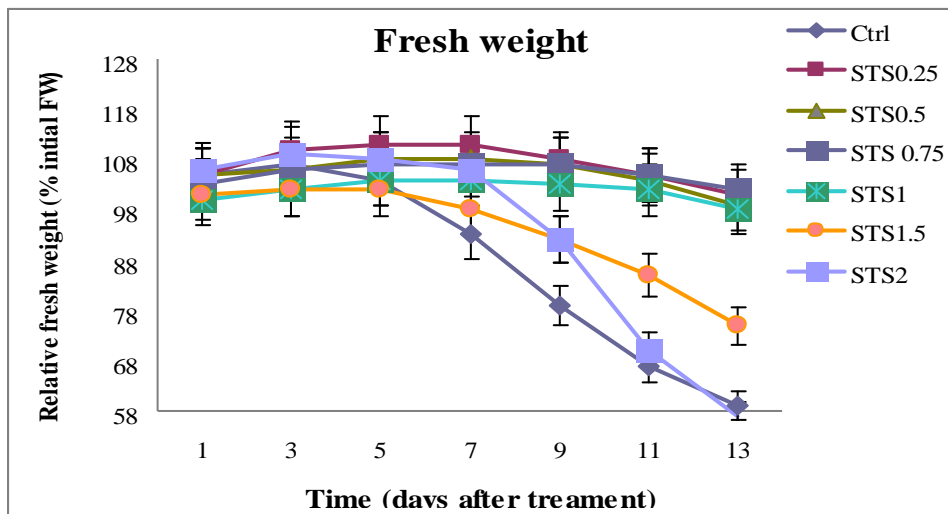
Fig.8. The effects of various concentrations of S-carvone and Humic acid on RFW of flowers.



**Fig.9.** The effects of different concentrations of *Crambe orientalis* L. extract on RFW of flowers.



**Fig.10.** The effects of various concentrations of Nano-Silver and Salicylic acid on RFW of flowers.



**Fig.11.** The effects of different concentrations of STS on RFW of flowers.

**THE YIELD AND QUALITY OF DIFFERENT *ELYMUS HISPIDUS* ACCESSIONS  
IN DRYLAND REGIONS**

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**Abstract**

Planting suitable species of forages can be a proper way to overcome feed shortages. For measuring the forage yield and quality traits of *Elymus hispidus*, 19 accessions of this plant were sown using randomized complete block design (RCBD) under dry land farming system. The 19 genotypes were grouped into 4 clusters based on Ward cluster analysis method. Collected data were analyzed for DM, plant height, stem number, leaf to stem ratio (LSR), dry matter digestibility (DMD), water soluble carbohydrate (WSC), crude protein (CP), acid detergent fibre (ADF) and ash. The results showed significant differences between genotypes for all of traits except LSR, CP and ash. Four genotypes (Khosh Yeelagh, Patava, Sabzkoh and Mimand) with average values of 4034, 3068, 2942 and 2450 kg ha<sup>-1</sup> had higher yield, respectively. Two genotypes (Khosh Yeelagh and Mimand) had higher quality and in terms of both yield and quality recognized as the best genotypes. There was positive correlation between DM with plant height and stem number. DMD showed positive and negative correlation with WSC and ADF, respectively. The relationship between CP and total ash was positively significant. The principal component analysis used to show variation between five quality traits (DMD, WSC, CP, ADF and ash). In the first component, four quality traits (DMD, WSC, CP, and ADF) determined about 76% of the total variations. Whereas DM yield and stem number were the important traits in the second components. The results showed that some genotypes of *Elymus hispidus* can adapt well to local climate conditions in dry regions.

**Key words:** *Elymus hispidus*, yield, quality, dry land, farming system

**Introduction**

*Elymus hispidus* species constitutes a varied group of perennial forage millet that has been extended in temperate areas of the world (Lawrence and Heinrichs, 1968). These plants, which are regarded as the most important rangeland millet of Iran, grow in cold steppe and temperate areas. This plant species with turf vegetative form has a considerable rangeland value (Mozaffarian, 2007). The native habitats of this species expand from steppes and mountain skirts of southern areas of Europe towards the Middle East, Central Asia and West Pakistan. It is regarded as a high yield plant in the areas with a maximum elevation of 3000 m from the sea level and an annual rainfall of 350 to 370 mm. Since Iran is one of the most important countries of forage plant variety, and has a very good potential for development of these product, therefore, for a better exploitation, a careful and appropriate planning is required so as to be able to preserve this scarce variety and exploit it (Mohammadi, 2006). The objective of this study was to introduce superior genotypes of the *Elymus hispidus*

species (forage quantitative and qualitative) to be use as suitable animal feed resoures, and is adaptable to local climatic conditions in order to determine the pattern of genetic variation and grouping the genotypes, using multivariate statistical methods.

### Materials and methods

In this study, the seeds of 19 genotypes of *Elymus hispidus* species, which were prepared from the gene bank of Natural Resources of Forest and Rangelands Research Institute, were grown at the rangeland plants station of Hossein Abad, 25 km to the west of Shiraz, in a completely random block design with 3 replicates in dryland conditions (totally 57 plots). After emergence of genotypes in the field, noting the traits in question before and after flowering was made. Weed weeding was made in mechanical form. At the end, the harvest of samples was made in two cuts, with a time interval of 30 days. For determination of the dry forage yield, the samples were weighed immediately after harvesting. After transferring to the lab, the samples were dried, re-weighed and the dry weight of the leaf, stem and leaf to stem ratio were determined. A sample of each genotype was grinded separately, and prepared for measuring the forage qualitative traits. The traits being investigated included the plant height, number of stems in each plant, leaf to stem ratio, and target yield, while the measurement of qualitative traits included the dry matter digestibility (DMD), crude protein (CP), water soluble carbohydrate (WSC), acid detergent fiber (ADF) and total Ash percentages, which were measured using the NIR system (Jafari and Naseri, 2007).

### Results and discussion

Analysis of variance and comparison between the means of genotypes for all quantitative and qualitative traits by Duncan method showed that there is variations among genotypes for the traits under study and one could utilize that variation in production of improved cultivars (Table 1).

Table 1- The sources and the means of the traits under study genotypes belonging to *E. hispidus* species in dry land conditions

No .	Genotype	Plant height	Number of stems	Leaf to stem ratio	Forage yield	Digestibility	Crude protein	Soluble sugars	ADF (%)	Total ash
1	Chahar Mahal	32.56 <sub>bcd</sub>	30.00 <sup>cd</sup>	3.00 <sup>a</sup> <sub>bc</sub>	2241 <sup>ed</sup>	43.73 <sup>abcde</sup>	17.90 <sup>a</sup>	11.86 <sub>cdef</sub>	44.20 <sup>b</sup> <sub>c</sub>	7.83 <sup>a</sup> <sub>b</sub>
2	Kohgilouy eh	34.89 <sup>b</sup> <sub>cd</sub>	41.89 <sub>abc</sub>	2.59 <sub>abc</sub>	3068 <sup>bc</sup> <sub>d</sub>	41.84 <sup>efg</sup>	18.09 <sub>a</sub>	10.52 <sup>g</sup>	46.22 <sup>a</sup> <sub>b</sub>	7.93 <sup>b</sup>
3	Esfahan	31.08 <sub>bcd</sub>	27.56 <sup>cd</sup>	2.44 <sub>abc</sub>	2489 <sup>ed</sup>	43.48 <sup>bcdef</sup>	17.67 <sub>a</sub>	12.61 <sub>abcdef</sub>	43.94 <sub>bcd</sub>	7.62 <sub>ab</sub>
4	Chahar Mahal	28.00 <sup>d</sup> <sub>c</sub>	42.89 <sup>ab</sup> <sub>c</sub>	2.57 <sub>abc</sub>	2450 <sup>ed</sup>	45.88 <sup>a</sup>	16.93 <sub>a</sub>	13.57 <sup>a</sup>	41.22 <sup>c</sup>	7.17 <sub>ab</sub>
5	Chahar Mahal	37.22 <sup>a</sup> <sub>bc</sub>	38.89 <sub>abc</sub>	2.91 <sub>abc</sub>	3615 <sup>ab</sup>	42.69 <sup>cdefg</sup>	17.19 <sub>a</sub>	12.17 <sub>bcd</sub>	44.87 <sup>a</sup> <sub>bc</sub>	7.43 <sub>ab</sub>
6	Fars	33.89 <sub>bcd</sub>	43.44 <sub>abc</sub>	2.69 <sub>abc</sub>	2485 <sup>ed</sup>	41.17 <sup>g</sup>	18.27 <sub>a</sub>	11.91 <sub>cdef</sub>	46.78 <sup>a</sup>	7.78 <sub>ab</sub>
7	Esfahan	26.89 <sup>d</sup>	37.00 <sub>abc</sub>	3.51 <sup>a</sup> <sub>b</sub>	2619 <sup>cd</sup> <sub>e</sub>	43.26 <sup>cdefg</sup>	18.20 <sub>a</sub>	12.48 <sub>abcdef</sub>	44.01 <sub>bc</sub>	7.48 <sub>ab</sub>

8	Kordestan	38.89 <sup>a</sup> <sub>b</sub>	18.11 <sup>d</sup>	2.38 <sup>b</sup> <sub>c</sub>	2772 <sup>bc</sup> <sub>de</sub>	41.38 <sup>fg</sup>	17.82 <sub>a</sub>	12.78 <sub>abcde</sub>	46.84 <sup>a</sup>	7.87 <sub>ab</sub>
9	Esfahan	35.00 <sub>bcd</sub>	34.56 <sup>bc</sup>	3.28 <sub>abc</sub>	2914 <sub>bcd</sub>	42.03 <sup>defg</sup>	17.21 <sub>a</sub>	11.59 <sub>efg</sub>	44.08 <sub>bc</sub>	7.33 <sub>ab</sub>
10	Kohgilouy eh	38.22 <sup>a</sup> <sub>b</sub>	38.22 <sub>abc</sub>	2.09 <sup>c</sup>	2942 <sub>bcd</sub>	45.66 <sup>ab</sup>	17.34 <sub>a</sub>	13.33 <sup>a</sup> <sub>b</sub>	41.46 <sub>de</sub>	7.32 <sub>ab</sub>
11	Kohgilouy eh	26.56 <sup>d</sup>	37.11 <sub>abc</sub>	3.13 <sub>abc</sub>	1918 <sup>e</sup>	43.91 <sup>abcde</sup>	17.13 <sub>a</sub>	11.43 <sub>fg</sub>	43.82 <sub>bcd</sub>	7.83 <sub>ab</sub>
12	Fars	36.67 <sup>a</sup> <sub>bc</sub>	39.22 <sub>abc</sub>	2.94 <sub>abc</sub>	2259 <sup>ed</sup>	43.86 <sup>abcde</sup>	17.68 <sub>a</sub>	11.63 <sub>defg</sub>	43.86 <sub>bcd</sub>	7.78 <sub>ab</sub>
13	Fars	29.78 <sub>bcd</sub>	31.56 <sup>cd</sup>	2.60 <sub>abc</sub>	1889 <sup>e</sup>	42.12 <sup>defg</sup>	17.47 <sub>a</sub>	12.89 <sub>abcd</sub>	44.67 <sub>abc</sub>	7.66 <sub>ab</sub>
14	Azarbaijan Gharbi	32.11 <sub>bcd</sub>	34.33 <sup>bc</sup>	3.26 <sub>abc</sub>	2219 <sup>ed</sup>	44.60 <sup>abc</sup>	17.10 <sub>a</sub>	12.73 <sub>abcde</sub>	42.76 <sub>cde</sub>	7.77 <sub>ab</sub>
15	Tehran	35.57 <sub>bcd</sub>	40.00 <sub>abc</sub>	3.27 <sub>abc</sub>	2366 <sup>ed</sup>	44.73 <sup>abc</sup>	17.60 <sub>a</sub>	12.97 <sup>a</sup> <sub>bc</sub>	42.73 <sub>cde</sub>	7.72 <sub>ab</sub>
16	Hamedan	30.33 <sub>bcd</sub>	42.33 <sub>abc</sub>	3.34 <sub>abc</sub>	2978 <sub>bcd</sub>	44.19 <sup>abcd</sup>	17.63 <sub>a</sub>	12.70 <sub>abcdef</sub>	44.71 <sub>abc</sub>	7.41 <sub>ab</sub>
17	Ardebil	45.33 <sup>a</sup>	33.00 <sub>bcd</sub>	3.81 <sup>a</sup>	2711 <sub>cde</sub>	43.76 <sup>abcde</sup>	17.21 <sub>a</sub>	12.37 <sub>abcdef</sub>	43.23 <sub>cde</sub>	7.67 <sub>ab</sub>
18	Mazandara n	34.22 <sub>bcd</sub>	52.73 <sup>a</sup>	3.33 <sub>abc</sub>	3484 <sup>ab</sup> <sub>c</sub>	42.77 <sup>cdefg</sup>	17.78 <sub>a</sub>	12.32 <sub>abcdef</sub>	44.93 <sub>abc</sub>	7.97 <sup>a</sup>
19	Golestan	37.11 <sup>a</sup> <sub>bc</sub>	47.56 <sup>ab</sup>	3.06 <sub>abc</sub>	4034 <sup>a</sup>	43.90 <sup>abcde</sup>	17.59 <sub>a</sub>	13.11 <sub>abc</sub>	43.73 <sub>bcd</sub>	7.23 <sub>ab</sub>

Non- similar letters in each column mean a significant difference between genotypes at 5% level by multi- range Duncan test. The findings of comparison between the means of traits showed that the genotypes of Khosh Yeylagh (Gorgan), Pataveh (Yassouj), Sabz Kooh (Chahar Mahal) and Meymand (Yassouj), with yields of 4034, 3068, 2942 and 2450 kg were the best genotypes, in that order, among which the genotypes of Khosh Yeylagh (Gorgan) and Meymand (Yassouj) had a better forage quality as well, which were proposed as appropriate genotypes for forage culture in the rangelands of the region. Generally, the findings showed that the genotypes of Gorgan (Khosh Yeylagh), with highest forage yield, plant height and stem concentration, had the highest content of soluble sugars as well. The genotypes of Noor-Baladeh, with higher yield, had a higher leaf to stem ratio, and, consequently, was more palatable. Perhaps the reason for being palatable and highly leafed of the latter species was the humid climate of north Iran. Next to these, the genotypes of Assad Abad (Hamedan) and Meymand (Yassouj), in addition to high forage yield, had a higher quality regarding the digestibility (Table 1).

The findings of the correlation coefficient showed that the forage yield, the plant height and the number of stems had a positive, significant correlation at 5% level. The results reported by Jafari and Naseri (2007), and Jafari and Rezaeifard (2010) are in line with the results of this study. The correlation coefficient between digestibility and soluble sugars was positive at 3% level, while it was negatively significant with ADF content. In other words, digestibility decreases with increasing ADF content.



### Conclusion

Some genotypes of *Elymus hispidus* can adapt well to local climate conditions and use as suitable livestock feed resources in dry regions. Planting and increasing the production of these species can reduce overgrazing pressure on the ranges, the output per each unit of feed intake will increase, and the conversion of feed to live weight gain can decrease.

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**THE INFLUENCE OF FOLIAR FERTILIZATION ON TOMATO LEAVES  
CHEMICAL CONTENT GROWN IN PROTECTED SPACES**

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**Abstract**

It was examined the impact of foliar fertilization with mineral fertilizers on the chemical content of leaves of tomatoes grown in protected spaces in terms of Strumica region. Experiment was set in six variants and three repetitions. The variants in the experiment were:

1. Control (untreated variant);
2. Chelan sol 11-4-42 + ME;
3. Folifertil 12-4-6 + ME;
4. Potassium nitrate 13-0-46;
5. Ariston 0-0-30;
6. Megagreen (CaCO<sub>3</sub> 82.3%, SiO<sub>2</sub> 5.56%, MgO 3.02%, CaO 41.7%, Fe 8783 mg/kg, Mn 156 mg/kg).

The experiment was set in 18 rows, and in each variant and repetition was included in 62 plants. During the vegetation were carried out seven treatments with following fertilizers at a concentration of 0.4%.

Before setting up the experiment agrochemical analyses of soil were performed and good fertility was determined with the nitrogen and phosphorus and medium fertility with potassium. Performed chemical analysis of tomato leaves showed that foliar fertilization had a positive impact. In the leaves of five variants with different fertilizers was found higher content of all tested parameters compared with the leaves of control untreated variant. The highest average phosphorus content (0.24%), potassium (0.71%) and zinc (0.017%) obtained in the leaves at variant 2.

The highest average calcium content (4.99%) and magnesium (0.67%) was determined in the leaves at variant 6.

The highest average iron content (0.018%) was determined in tomato leaves of variant 2 and variant 6.

**Key words:** mineral fertilizers, tomato, leaves

## Introduction

For normal growth and development of crops of great importance is optimal plant nutrition. Plant nutrition affects a number of physiological and biochemical processes that take place in different organs. Each nutrient element has its specific impact on certain parts of the plant.

Tomato is a one-year crop with great economic importance. Fruits are consumed fresh as well as various preparations (Lazic Branka et al., 2001; Tudzarov, 1990). Fruits are characterized by high nutritional value. It is rich in many vitamins, organic and mineral substances. It has high technological value (Vujanovic, 2008; Branka Lazic, 1990). As a result of cultivation in protected spaces can be used throughout the year.

In certain unfavorable climatic and soil conditions, such as insufficient amount moisture, low pH, or unfavorable soil structure, a difficult passage of nutrients through the root system. Thus the plant is sufficiently equipped with the necessary macro and micro elements that affect the quality and quantity of yields (Maksimovic and Jain Nada, 1996; Nenadov, 1985).

In order to achieve good quality and quantity of tomatoes essential is the foliar fertilization.

The leaf is an organ in which the synthesis of organic matter takes place at most. The composition of leaf tissue and symptoms that occur in it are the best indicators for determining the level of nutrients in the soil, and thus the need for fertilization (Jekic and Brkovic, 1986; Saciragic and Jekic, 1988). The chemical composition of the leaves is variable. The presence of certain nutrients in the leaves depends on the stage of taking a leaf samples, the type of plant, mineral nutrition, conditions of cultivation.

In foliar nutrition, nutrients quickly come to chloroplasts where photosynthesis takes place and other physiological and biochemical processes. With faster foliar nutrition prevents deficiency in certain elements that occur in the leaves (Takac, 2009).

The aim of our exploration was to determinate the influence of foliar fertilizing with different mineral fertilizers on tomato leaves chemical content grown in protected spaces in Strumica area.

## Material and method

In the Strumica area in the vicinity of the v. Kuklis during the year of 2008 and 2009 the experiment was placed in the protected space of 300 m<sup>2</sup>.

Experiment was set in 18 rows. In tests were involving six variants in 3 repetitions.

Material for the work was the tomato's variety *bele*. The seedling was planted in rows with row by row distance 60 cm, and between plants 40 cm. The experiment was set in conditions of watering. During the vegetation period of tomatoes were applied basic agro-technical measures. Before seeding planting was made soil fertilization with mineral fertilizer NPK 6-10-30 + 2% MgO in the amount of 12 kg in the hall with an area of 300 m<sup>2</sup>.

Variants in the experiment were:

1. Control (untreated variant);
2. Chelan sol 11-4-42 + ME;
3. Folifertil 12-4-6 + ME;
4. Potassium nitrate 13-0-46;
5. Ariston 0-0-30;
6. Megagreen.

In each variant and repetitions were included in 62 plants, and total for the entire experiment there were 1116 plants.

Each variant was treated with tasted foliar fertilizer in concentration of 0.4% solution. The application of fertilizers was done with hand spray, by spraying the played leaves. During the

vegetation were conducted seven foliar treatments, starting from the stage of growth of the first fruits.

Five types of mineral fertilizers were used:

1<sup>st</sup> Chelan sol 11-4-42 + ME (Fe 0.01%, Mn 0.01%, Cu 0.01%, Co 0.001%, Mo 0.001%);

2<sup>nd</sup> Folifertil 12-4-6 + ME (Fe 0.01%, Mn 0.01%, Cu 0.01%, Co 0.001%, Mo 0.001%);

3<sup>rd</sup> Potassium nitrate 13-0-46;

4<sup>th</sup> Ariston 0-0-30;

5<sup>th</sup> Megagreen (CaCO<sub>3</sub> 82.3%, SiO<sub>2</sub> 5.56%, MgO 3.02%, CaO 41.7%, Fe 8783 mg/kg, Mn 156 mg/kg).

After completion of harvesting leaves were taken separately for variants that are specified following parameters:

Content of phosphorus (P<sub>2</sub>O<sub>5</sub>) determined using atomic emission spectrometry with inductively coupled plasma (ICP - AEC) (Saric et al., 1986);

Content of potassium (K<sub>2</sub>O) determined by incineration of the material with concentrated H<sub>2</sub>SO<sub>4</sub> and its determination plamenfotometar (Saric et al., 1986);

Content of calcium (SAT) determined using atomic emission spectrometry with inductively coupled plasma (ICP - AEC) (Saric et al., 1986);

Content of magnesium (Mg) determined by applying atomic; emission spectrometry with inductively coupled plasma (ICP - AEC) (Saric et al., 1986);

Content of iron (Fe) determined using atomic emission spectrometry with inductively coupled plasma (ICP - AEC) (Saric et al., 1986);

Content of manganese (Mn) determined using atomic emission spectrometry with inductively coupled plasma (ICP - AEC) (Saric et al., 1986);

Content of zinc (Zn) determined using atomic emission spectrometry with inductively coupled plasma (ICP - AEC) (Saric et al., 1986).

Before setting up the experiment soil samples were taken for agrochemical and analyses were performed on the following parameters:

pH - Reaction determined potentiometric with pH meter (Bogdanovic et al., 1966);

Content of easy available nitrogen – chosen by method of Tjurin and Kononova;

Content of easy available phosphorus – chosen by AL method and reading of spektrofotometer (Bogdanovic et al., 1966);

Content easy available potassium – chosen by AL method and reading of spektrofotometer (Bogdanovic et al., 1966);

Content of carbonates – chosen with Schaiblerov Calcimetar (Bogdanovic et al., 1966).

### **Results and discussion**

The content of macro and micro elements in tomato fruits, among others, largely depends on soil fertility.

To achieve high and quality yields in protected spaces tomatoes requires favourable soil and climatic conditions (Glentic and Krstic, 1990).

Quality tomatoes are getting deep and loose soil rich in easily available nutrients. The optimum soil pH reaction for tomatoes is weakly acidic with a pH value of 5.5 to 6.0 (Lazic Branka, 1990).

Table 1. Agrochemical soil analysis

Order No.	Plot	Depth (cm)	pH		Available forms (mg/100 g soil)			CaCO <sub>3</sub> (%)
			H <sub>2</sub> O	KCl	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	
1	Tomato I part	0-20	7.35	6.65	8.50	24.60	17.90	/
2		20-40	7.40	6.64	7.80	25.80	16.50	/
<b>Average</b>		<b>0-40</b>	<b>7.37</b>	<b>6.64</b>	<b>8.15</b>	<b>25.20</b>	<b>17.20</b>	/
3	Tomato II part	0-20	7.43	6.70	8.60	25.70	17.50	/
4		20-40	7.40	6.60	7.80	25.60	17.80	/
<b>Average</b>		<b>0-40</b>	<b>7.41</b>	<b>6.65</b>	<b>8.20</b>	<b>25.65</b>	<b>17.65</b>	/

From the data in Table 1 can be concluded that soil which is set experiment had a neutral pH, good fertility with nitrogen and phosphorus, and potassium fertility medium available. There was no presence of carbonates.

From the data in Table 2 can concluded that foliar fertilization had a positive influence on the content of the examined macro and micro elements in tomato leaves. In all variants analyzed parameters had higher content, compared to the control untreated variant.

The highest average phosphorus content (0.24%), potassium (0.71%) and zinc (0.017%) was determined in tomato leaves of variant 2.

According the content of phosphorus, statistically significant differences were obtained on the two tasted levels in variants 2, 3 and 6. According the content of potassium statistically significant difference was obtained on the level 0.05 in the variants 2 and 5 and on the level 0.01 in the variant 2.

The highest average calcium content (4.99%) and magnesium (0.67%) was determinate in the variant 6. Statistically significant differences were obtained for the content of calcium level 0.05 in the variant 2, 4, 5 and 6 and on the level 0.01 in the variant 6.

The highest average iron content (0.017%) was determinate in the leaves from the variant 2 and 6. No statistically significant differences obtained.

Higher content of tested elements in the leaves of treated variants, compared to the control variant leaves was as a result to the chemical composition of used foliar fertilizers. Examined fertilizers contain macro and micro elements present in different ratio. With foliar fertilization enables rapid absorption of nutrients in the leaves of the plant. Absorbed elements are transported to other organs of the plant. In this way foliar fertilization allows the plant to be supplied with the necessary nutritional elements.

Table 2. Content of P<sub>2</sub>O<sub>5</sub> K<sub>2</sub>O, Ca, Mg, Fe and Zn in % of dry matter average 2008/2009

Variant	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	Ca	Mg	Fe	Zn
1	0.11	0.58	2.42	0.43	0.017	0.016
2	0.24	0.71	2.49	0.48	0.017	0.017
3	0.17	0.64	2.59	0.46	0.017	0.016
4	0.11	0.65	2.45	0.47	0.017	0.016
5	0.11	0.67	2.61	0.47	0.017	0.016
6	0.17	0.57	4.99	0.67	0.017	0.017

LSD 0.05=0.031    LSD 0.05=0.076    LSD 0.05=0.038    LSD 0.05=0.032    LSD 0.05=7.604    LSD 0.05=0.527  
LSD 0.01=0.042    LSD 0.01=0.102    LSD 0.01=0.051    LSD 0.01=0.044    LSD 0.01=10.305    LSD 0.01=0.715

### Conclusion

According to the results for the influence of foliar fertilization over the content of macro and micro elements in tomato leaves grown in protected spaces can be made the following conclusions:

Soil where the experiments were placed is characterized by good fertility with easily available nitrogen and phosphorus, and easily available potassium medium fertility;

In all variants where the foliar fertilizers were used, were obtained higher content of the studied elements compared with leaves from the control variant;

The highest content of phosphorus, potassium and zinc was obtained in the leaves of variant 2 Chelan sol 11-4-42 + ME (Fe 0.01%, Mn 0.01%, Cu 0.01%, Co 0.001%, Mo 0.001%);

The highest average content of calcium and magnesium was obtained in the leaves of variant 6 Megagreen (CaCO<sub>3</sub> 82.3%, SiO<sub>2</sub> 5.56%, MgO 3.02%, CaO 41.7%, Fe 8783 mg / kg, Mn 156 mg / kg);

The highest average iron content was obtained in the leaves of variant 2 Chelan sol 11-4-42 + ME (Fe 0.01%, Mn 0.01%, Cu 0.01%, Co 0.001%, Mo 0.001 %) and lime. 6 Megagreen (CaCO<sub>3</sub> 82.3%, SiO<sub>2</sub> 5.56%, MgO 3.02%, CaO 41.7%, Fe 8783 mg / kg, Mn 156 mg / kg);

Statistically significant differences were obtained in all of the studied elements except for iron content.

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## A STUDY OF BASIL TYPES IN THE COASTAL PLAINS OF ALBANIA

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### Abstract

Basil is a plant of Lamiaceae family, with wide spectrum use in food industry, perfumery, as fresh spice, flavoring different environments, as well as in medicine. This is why basil is considered both a spice and a medical herb. These values are resulted from high content of ocimol in all plant organs (leaves, flowers, fruits, seed and roots. The fact that Dioscorides mentions that herb early in the first century, as a medicinal plants for the disinfection of premises, mouth and teeth, shows the values and its recognition since Antiquity. In Albania it is a known and cultivated plant, in families, gardens, and it has synonyms by area. For essence production, it begins to be cultivated in the 60s and, nowadays the demand is growing. Basil studies are limited in technology and comparisons of subspecies and varieties. A study of five subspecies in coastal plains of Albania (Toshkëz-Lushnja) is presented in this paper. The differences found are statistically necessary.

**Keywords:** essence, spice, the cultivation

### Materials and methods

This study encompassed the five types of basil. The seeds have been brought from Italy and they are as follows:

Limonez

Napolitan

Red basil

Fino Verde

Genoves basil

The experiment was set up according to the randomized block scheme, with five variants and four repetitions with variant size of 28m<sup>2</sup> (2.4 m x 11.7 m).

The experiment was set up in Toshkez - Lushnja, according to randomized block scheme, as the methodology had provided. The chemical composition of the soil is: humus 0.7%, pH 7.55, nitrogen 0.18%, phosphorus 17.7 %, potassium 12.5 ppm and calcium 9.07 ppm.

Scheme No. 1. The experiment set up.

2	1	3	4	5
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4	5	3	2	1
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1	2	4	5	3
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1	3	4	5	2
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There was mowing in the full flowering phase by making the separation in accordance of the organ:

Fresh herbs (blooms and leaves)

Fresh stalks

Fresh mass

Drying was performed in the shade, separately for each body, up to 10% humidity and there have been identified the weighing for the three organs:

Dry herbs (blooms and leaves)

Dry stalks

Dry mass

These are done separately for the three scythes, and have become amounts to three scythes and the three organs, and the total, too (herbs + stalks + dry and fresh mass).

### Results and discussion

Table 1. Inter-distances of saplings

No.	Types	Inter-distance	No. of plants	Area m <sup>2</sup>
1	Limonez	60 x 50	88	28
2	Napolitan	60 x 45	105	28
3	Red basil	60 x 45	106	28
4	Temines (Fino verde)	60 x 55	85	28
5	Genoves	60 x 55	88	28



### The preparation of saplings and planting in the field

The saplings are produced in greenhouses with central heating by sowing seeds in polysterol seed pans, with 250 holes, placing two seeds for each hole. Before planting the seeds are placed in warm water, to provoke their uniform and completed germination. Seeds are planted at a depth 0.3 - 0.5 cm. In every 3-4 hours it is realized automatically irrigation, with the amount of water 10 mm. During vegetation there were made fertilization, for 52 days sapling was ready for planting in the field. The soil tillage is made in September 2010, at 31-35 cm depth. In the spring it is carried the soil milling in 12-15 cm depth. Planting in the field is realized with workforce. Planting is done with pins in the inter-distances provided in table 1.

Table 2. The weight of fresh herbs (g)

Nr	The types of basil		Mowings			Sum K (1-3)
			I	II	III	
1	Limonez		5712,5	15595	3106,25	24413,75
2	Napolitan		5407,5	12727,5	2488,75	20623,75
3	Red basil		2107,5	4755	878,75	7741,25
4	Fino verde		2790	6007	1385	10182
5	Genoves		2355	8972,5	1557,5	12885
	Dmv	0,05	349,2	680,3	286,85	786,8
		0,01	408,1	747,5	324,13	842,6

The herbs are all plant organs that are used for many purposes and they take the brunt of basil production. Among the types of basil in production there have been some changes observed that testify to the morphological characteristics and plant development. . The higher production of herbs is taken from Limonez basil, following by Napolitan basil. Between the mowing there are differences in production, where the second mowing gives the highest production for all types of basil. The herb of Fino-verde basil is in small amounts, but it is rich in essence and it finds wider use, especially in the production of tomato sauce. Just, for this reason it is introduced in the study field.

Table 3. The weight of fresh stalks (g)

Nr	The types of basil		Mowings			Sum K (1-3)
			I	II	III	
1	Limonez		3242,5	8107,5	1630	12980
2	Napolitan		2927,5	8392,5	1306,25	12626,25
3	Red basil		892,5	1300	468,75	2661,25
4	Fino verde		787,5	1920	717,5	3425
5	Genoves		763,75	2822,5	815,7	4401,95
	Dmv	0,05	311,7	522,4	154,3	934,6
		0,01	384,5	578,5	176,8	1021,8

The stalk production represents differences between the basil types. Limonez basil and Napolitan basil give the highest production of the stalks. So, at Fino Verde it is observed that the stalk production is three times higher than the herb production.

Table 4. The weight of fresh mass

No	The types of basil		Mowing			Sum K (1-3 )
			I	II	III	
1	Limonez		8955	23702,5	4736,25	37393,75
2	Napolitan		8335	21120	3795	33250
3	Red basil		3000	6055	1347,5	10402,5
4	Fino verde		3577,5	7972	2102,5	13607
5	Genoves		3118,75	11795	2373,2	17286,95
	Dmv	0,05	642,4	1326,41	456,4	1567,7
		0,01	695,6	1418,52	528,7	1634,8

Even the fresh mass production presents the verified differences between the basil types. Limonez and Napolitan basils achieve the highest production.

Table 5. The weight of dry herbs (g) (leaves and herbs).

No	The basil type		Mowing			Sum K (1-3 )
			I	II	III	
1	Limonez		1054,5	3106,25	515,75	4676,5
2	Napolitan		1270	2488,75	436,25	4195
3	Red basil		545	883,75	166,75	1595,5
4	Fino verde		698,5	1385	240,75	2324,25
5	Genoves		507	1558,75	269,5	2335,2
	Dmv	0,05	118,2	226,7	48,75	382,8
		0,01	136,4	298,4	62,36	416,4

The performance of the dried leaf mass presents the same nomocracy as to fresh leaf, but Fino-verde basil gives a small proportion to the fresh and dry mass, which goes up to 3-4:1.

Table 6. The weight of dry stalks (g)

No	The basil type		Mowing			Sum K (1-3 )
			I	II	III	
1	Limonez		762,5	2868,75	527,5	4176,75
2	Napolitan		880	1836,25	417,5	3133,75
3	Red basil		255	527,5	155	937,5
4	Fino verde		256,25	901,25	181,5	1339
5	Genoves		215	1257,5	255	1727,5
	Dmv	0,05	64,7	189,7	442,4	328,92
		0,01	95,8	226,4	60,75	408,74

The weight of dry stalks presents differences between the basil types, where the highest production is achieved by Limonez basil, following by Napolitan basil, but with a great difference with three other types of basil.

Table 7. The weight of dry mass (g)

No	Te basil types	Mowing			Sum K (1-3 )	
		I	II	III		
1	Limonez	1817	5975	1044,75	8836,75	
2	Napolitan	2150	4325	853,75	7328,75	
3	Red basil	800	1411,25	321,75	3065	
4	Fino verde	954,75	2286,25	2407,5	5848,5	
5	Genoves	722	2816,25	524,5	4062,75	
	Dmv	0,05	127,6	411,3	129,7	326,4
		0,01	164,3	486,4	193,82	409,53

The production of dry mass follows the same nomocracy as to the fresh mass. The highest production is achieved by Limonez basil, the second is Napolitan basil, with a great difference with the three other types.

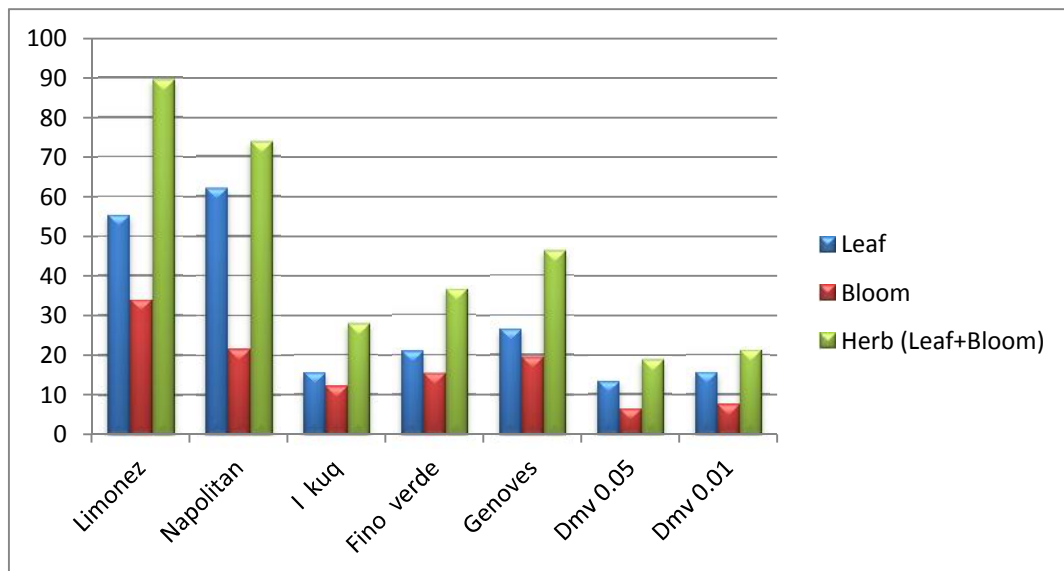


Chart 1. The fresh mass yield (q/ha)

Analysis of the performance of the fresh mass highlights that Neapolitan and Limonez basil provide higher production and are included in the same group for Dmv 0:05 and Dmv0:01 are differentiated. So, Limonez basil gives the greatest amount of fresh herb.

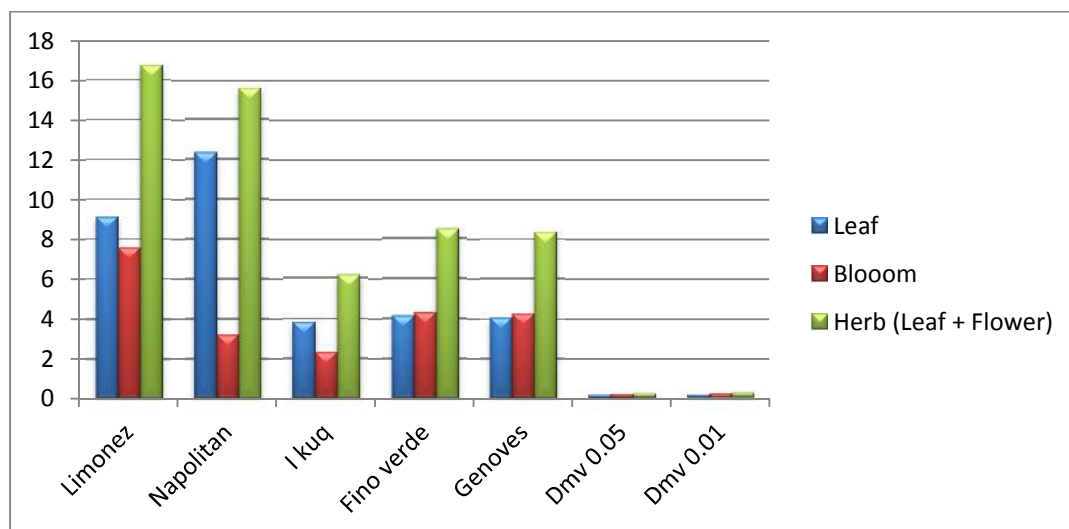


Chart 2. The yield of dry mass (q/ha)

The performance of the dry herb presents differentiations, drawing in the first place only Limonez subspecies. This is because the leaf of Napolitan basil is pulp and it has the highest content of moisture, increasing the dry mass/ fresh mass ratio.

### Conclusions

From the second year of the study can derive some conclusions:

Among the subspecies are been marked differences in the yield of fresh mass and the plant organs.

The dry mass of plant organs presents the major differences that affected by the ratio between the fresh mass and dry mass, which is different for subspecies of basil.

The fresh mass and dry mass ratio is between 3.4 : 1 at Fino Verde basil, till 4.4 : 1 at Limonez basil

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## INFLUENCE OF APPLIED AGRICULTURAL MEASURES ON THE SEEDLING QUALITY OF LETTUCE

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### Abstract

In the region of Zeta and BjelopavliCi mostly two varieties of lettuce are cultivated: cabbage lettuce (*Lactuca sativa var. capitata*) and leaves of lettuce (*Lactuca sativa var. Acephala*). With a goal to determine the influence of time of sowing (3.VII and 1.VIII), fertilizers (Slavol, WUKSAL super 8:8:6+me i Poly-Feed MAR 20:20:20+me) and substrates (Profi-substrat i Blumenerde) on a cultivation of lettuce, the experiment has been set up in the plain of Zeta (in the region of Podgorica). The examined parameters were the beginning of germination, the percentage of emerged plants, the time of the first leaf appearance, rosette leaf number at the time of planting, the mass of the plant seedling.

By using the microbiological fertilizers one gets the better germination and the earlier springing up of lettuce plantations. The seed of the Nadine F1 lettuce, treated with Slavol before sowing, has sprung up two days earlier than the seed that hasn't been treated at all. The plants of lettuce plantations have big needs for nutrients because of the intensive growth, therefore the higher yield has been gained by using the fertilizers that provide more nutrients. Blumenerde substrat has, in the both times of sowing, showed better results than the Profi-substrat, where the mass of a plantation was 1,97g i 2,19g.

**Key words:** lettuce, Nadine F1, times of sowing, fertilizer, substrat

### Introduction

Lettuce is an annual vegetable crop with the spindle, well-developed root which mainly develops in the surface layer of soil, and especially shallow rooted lettuce produced from seedlings. The stalk of lettuce in the vegetative stage has shortened internodes that gush leaves forming a rosette. The leaves are sessile, wide, relatively thin, with soft oily tissue. The outer leaves of the rosette were horizontal and darker, while the leaves of head are vertical and brighter in color (*Prohens and Nuez, 2008*).

The seedling is a plant from germination to the formation of 4 to 10 permanent leaves or passing four or five stages parthenogenesis *Markovic (2002)*. According to the same author, the seedlings provides an earlier harvest and better use of space. Plant height depends on the quality of seedlings, leaf arrangement, root widespread, flowering and amount of yield.

According *Para ikoviC et al. (2007)*, germination of seeds is affected by heat, humidity, soil pH and the presence of the organic and inorganic components in growing media, and the optimum temperature during germination and emergence are between 15-20°C. Adoption of some elements depend on environmental factors, developmental stage of the plant and the genotype (*Djuric et al., 2005*).

Application of biofertilizers contribute to the stability and quality of yields, preserve the ecological balance, which reflects on the food safety and a favorable economic effect (*Milosevic et al., 2003*). Treatment of seeds and of seedlings of vegetable crops with a Slavol

has affected growth of seedlings height and dry matter, as well as increased adoption of nutrients from the soil (*Djordjevic et al., 2004; Simic et al., 2005*).

The highest quality seedlings are produced in containers (*Filkovic et al., 2009*) because the root is not intergrowths and sufficient aeration of the substrate stimulates the development of lateral roots, *Markovic (2002)*.

Lettuce (*Lactuca sativa L.*) is a vegetable with low energy value (18 cal/100g fresh eatable part). The water content is up to 94%, carbohydrates 2.9%, 1.2% protein and 0.2% fat. In the 100g fresh lettuce, by *Tranevski (2008)*, is vitamin C (10mg), B1 (0.08 mg), B2 (0.12 mg), B6 (0.2 mg), PP (0.6 mg), carotene (1 mg) and vitamin E (0.6 mg).

According to *Prohens and Nuez (2008)*, the objectives to further lettuce breeding need to be developed in three directions: resistance to pests and diseases, increasing of yields and improvement of quality.

### **Materials and methods**

In order to determine the impact of agrotechnical measures applied to lettuce seedlings Nadine F1 experiments have been conducted in the region of Zeta (near Podgorica). The experiment was set up as a three factorial and examined factors were sowing time (term), fertilizer and substrate. The experiment was conducted by split-plot design with three repetition. Four weeks after sowing, from each repetition has been taken five plants on which we have measured mass of the whole plant and roots of seedlings.

Sowing was done on 3th VII and 1st VIII 2009 in containers with a 126 apertures (volume 22 cm<sup>3</sup>). The examined fertilizers were Slavol (nitrogen fixing and phosphate mineralizing bacteria, natural vitamins, enzymes and growth stimulants Agrounik-doo, Belgrade), Poly-Feed MAR 20:20:20 + I (Haifa Chemicals Ltd., Israel) and WUKSAL Super 8: 8:6 + I (Chemical Agrosava).

Treatment of Slavol consisted of pre sowing seed treatment with a 33.3% solution and feeding seedlings old twelve and seventeen days with a 1% solution. WUKSAL is applied foliar twelfth and seventeenth day in the concentration of 1%. Treatment of Poly-Feed MAR was performed twelfth and seventeenth day with 0.5% solution. Control is irrigated with water without any fertilizers.

Examined substrates were Profi-substrate (GRAMOFLOR) and Blumenerde (NATURAHUM). Profi-substrate composition was 60% white peat, black peat 40% and a pH value of 5.2 to 6.0. Blumenerde substrate composition is 100% white peat and pH 5.4 to 6.0.

The influence of observed factors was assessed by monitoring of the start of germination, the percentage emerged plants and timing of the first full leaf, the number of rosette leaves at the time of planting, the mass of the whole plant and root mass of lettuce seedlings. The influence of the applied measures was analyzed using the F-test.

### **Results and discusion**

In the production of seedlings should be used very high quality seeds, high biological value (high germination and vigor) (*Markovic, 2002*). Larger number germinated seeds and emerged plants is potentially larger number of plant of seedlings. With faster germination can be achieved shorter period of seedling production. The seeds treated before sowing by Slavol has begun to sprout second day after seeding, while the seeds of the other treatments began springing fourth day after sowing. Recorded results are consistent with the allegations of *Djordjevic et al. 2004*.

Table 1. Time and the percentage of germination and time to the first full leaf depending on planting date, type of substrate and type of fertilizer

Cultivar	Inoculation by Slavol	Substrate	Days from sowing to emergence		% emergence		The appearance of the first full leaf	
			I term	II term	I term	II term	I term	II term
Nadine F1	Inoculated	Profi-substrat	2	2	100	77	6	6
		Blumenerde	2	2	92,9	77	6	6
	Not inoculated	Profi-substrat	4	4	85,47	75,5	7	8
		Blumenerde	4	4	88,89	72,2	7	8
<i>Average</i>					<b>91,82</b>	<b>75,43</b>		

	Sowing time	Fertilizer	Substrate	Sowing time / Fertilizer	Sowing time / Substrate	Fertilizer / Substrate	Sowing time / Fertilizer / Substrate
F calculated (% emergence)	<b>18,4**</b>	2,38	0,367	0,924	0,012	0,124	1,14

Sowing date was statistically significant effect on the percentage emerged plants.

On plants treated by Slavol appearance of the first full leaf is spotted 6 days after sowing (in both term). In the other treatments, the plants have formed the first full leaf 7 days after the seeding (first sowing term), and 8 days after the seeding in the second term.

Table 2. Number of leaves of seedlings depending on sowing date, type of substrate and type of fertilizer

Cultivar	Fertilizer	Substrate	Number of leaves at the time of planting		Average	Average
			I term	II term		
Nadine F1	Slavol	Profi-substrat	6,6	6,6	6,6	6,9
		Blumenerde	7,4	7,0	7,2	
	WUKSAL super	Profi-substrat	6,2	7,4	6,8	7,1
		Blumenerde	7,0	7,6	7,3	
	Poly-Feed MAR	Profi-substrat	6,0	7,0	6,5	6,8
		Blumenerde	7,2	6,9	7,1	
	Control	Profi-substrat	5,8	6,4	6,1	6,4
		Blumenerde	6,2	7,2	6,7	
<i>Average</i>			<b>6,55</b>	<b>7,01</b>		

	Sowing time	Fertilizer	Substrate	Sowing time / Fertilizer	Sowing time / Substrate	Fertilizer / Substrate	Sowing time / Fertilizer / Substrate
F calculated	<b>9,48**</b>	<b>4,14**</b>	<b>14,5**</b>	<b>3,53*</b>	3,97	0,120	2,22

All three examined factors had a significant effect on number of leaves, and the interaction of sowing date / fertilizer significant effect on the same characteristic. The number of formed leaves at the time of transplanting was higher in the second sowing date compared with the first sowing date. The lowest average number of leaves was observed in the control (6.4 leaves/plant), while the highest number of leaves was recorded in the treatment with WUKSAL and get better results pointed at Blumenerde substrate. Mass of plant seedlings directly depends on the leaves number formed until the moment of transplanting.



Table 3. Mass of plant lettuce seedlings depending on the sowing date, type of substrate and type of fertilizer

Mass of plant lettuce seedlings (g)							
Fertilizer	Profi-substrat			Blumenerde			Average
	I term	II term	Average	I term	II term	Average	
Slavol	1,90	1,52	1,71	2,20	2,52	2,36	2,04
WUKSAL super	1,23	1,33	1,28	1,82	1,72	1,77	1,53
Poly-Feed MAR	2,11	1,47	<b>1,79</b>	2,49	2,51	<b>2,50</b>	<b>2,15</b>
Control	1,20	0,68	0,94	1,37	1,99	1,68	1,31
<i>Average</i>	<i>1,61</i>	<i>1,25</i>		<i>1,97</i>	<i>2,19</i>		

	Sowing time	Fertilizer	Substrate	Sowing time / Fertilizer	Sowing time / Substrate	Fertilizer / Substrate	Sowing time / Fertilizer / Substrate
F calculated	0,468	<b>15.3**</b>	<b>39.6**</b>	<b>39.6**</b>	<b>7.82**</b>	0.307	1.88

In our studies, statistically significant effect on seedlings plant mass had factors: fertilizer and substrate, interaction sowing date / fertilizer, as well as the interaction sowing date / substrate. Blumenerde substrate in both sowing date gave better results than the Profi-substrate, and the average mass of seedlings was 1.97g and 2.19g. Treatment with Poly-Feed MAR fertilizer showed best results in the first sowing date (2.30g) on the both substrates, and treatment with Slavol in the second sowing date for both substrates (2.02g). At seedlings treated with Poly-Feed MAR detected the largest plant mass on the both substrates (1.79g and 2.50g).

Table 4. The root mass seedlings lettuce depending on the sowing date, type of substrate and type of fertilizer

The root mass seedlings lettuce (g)							
Fertilizer	Profi-substrat			Blumenerde			Average
	I term	II term	Average	I term	II term	Average	
Slavol	0,16	0,17	0,17	0,16	0,77	0,47	0,32
WUKSAL super	0,14	0,21	0,18	0,19	0,54	0,37	0,27
Poly-Feed MAR	0,44	0,43	<b>0,44</b>	0,38	0,67	<b>0,53</b>	<b>0,48</b>
Control	0,15	0,25	0,20	0,23	0,36	0,30	0,25
<i>Average</i>	<i>0,22</i>	<b>0,27</b>		<i>0,24</i>	<b>0,59</b>		

	Sowing time	Fertilizer	Substrate	Sowing time / Fertilizer	Sowing time / Substrate	Fertilizer / Substrate	Sowing time / Fertilizer / Substrate
F calculated	1.88	<b>12,6**</b>	<b>32,1**</b>	2,26	<b>25,3**</b>	<b>2,78*</b>	<b>3,75*</b>

At root mass, similarly to the mass of of the whole plant lettuce seedlings, a highly significant effect had fertilizer and the substrate, and the interaction sowing date / substrate. Interactions of fertilizer / substrate and sowing date / fertilizer / substrate significantly affected the mentioned characteristic.

Seedlings treated with Poly-Feed MAR had significantly greater root mass in both sowing date on both substrates, compared to treatment with other fertilizers. In the second sowing date on the both substrates were recorded better results in comparison to the first sowing date.

Table 5. *F* test for examined treatments and the characteristics of lettuce seedlings

Sources of variation	F calculated			
	Mass of plant lettuce seedlings	The root mass seedlings lettuce	% emergence	Number of leaves at the time of planting
A	0,468	1,88	<b>18,4**</b>	<b>9,48**</b>
B	<b>15,3**</b>	<b>12,6**</b>	2,38	<b>4,14**</b>
C	<b>39,6**</b>	<b>32,1**</b>	0,367	<b>14,5**</b>
A x B	<b>39,6**</b>	2,26	0,924	<b>3,53*</b>
A x C	<b>7,82**</b>	<b>25,3**</b>	0,012	3,97
B x C	0,307	<b>2,78*</b>	0,124	0,120
A x B x C	1,88	<b>3,75*</b>	1,14	2,22

Factor A- Sowing date (term); Factor B- Fertilizer; Factor C- Substrate

*Djordjevic et al. (2004)* suggest that the selection of quality seeds is very important, and our seed by Nadine F1 in certain treatments showed excellent germination of 100%. *Markovic (2002)*, the length of emergence is 3-6 days, the seeds that were treated with Slavol has sprung up after 2 days, and other treatments after 4 days.

During the first and second term of growing seedlings, mean daily temperatures were higher than 25°C, and *Para ikoviC et al. (2007)* suggest that such a temperature in the lettuce can cause secondary dormancy, postpone germination and reduced germination percentage of the seeds germinated. In the second term of growing temperatures were average higher by 1.1°C than in the second term and accordingly with that percentage of seedlings in the second term was lower average 17.9%.

Plants of lettuce seedlings has a great need for nutrients because of a short growing season and intense growth (*Markovic, 2002; Djuric et al., 2005*) and better results were obtained by applying fertilizers that provide more nutrients. Microbial fertilizers can be used as a replacement or supplement of mineral fertilizers (*Illmer et al., 1995; Djordjevic et al., 2005; Simic et al., 2005; Govedarica et al., 2002*). In our experiment, all fertilizers gave better results than the control. *Jarak et al. (2004)*, the abundance and activity of microorganisms was higher in soils and substrates with good structure, favorable aeration and moisture and neutral pH value. The optimum of pH of substrate for the most bacteria is 6-7,5 (*Loncaric et al., 2005*), that we provided the Blumenerde and Profi-substrate (5.2 to 6.0). Mass of plant lettuce seedlings treated with Slavol (containing *Bacillus subtilis* and *Bacillus megaterium var. Phosphaticus*) was higher for 35.8% and root mass of for 21.9% compared to the control. According to the *Djordjevic et al. (2004)*, by treating the the seed of onion with *Bacillus subtilis* was greater root mass for the 13-100%, and treating the land by bacteria *Bacillus megaterium var. phosphaticus* was increased tomato yield for 35%.

In accordance with the recommendation of *Vavrina (1998)*, containers with 126 apertures are suitable for the production of lettuce seedlings and the seedlings ready for planting after a period of 20-30 days from sowing, *Markovic (2002)*.

### Conclusions

The research results of agricultural practices influence on the quality of lettuce showed that:

Examined factor sowing date:

- No effect on lettuce seeds germination time.
- The percentage of seedlings was significantly higher in the first sowing date.
- Before sowing treatment with Slavol has accelerated emergence of the first leaf in both sowing date.
- Number of leaves at the moment of transplanting was significantly higher in the second sowing date.
- The maximum mass of plant and roots was recorded in the second sowing date.

Examined substrate factor:

- No effect on lettuce seeds germination time.
- The percentage of seedlings was higher in the Profi-substrate
- The appearance of the first full leaf is not dependent of the substrate.
- There has been a significantly higher number of leaves on the Blumenerde substrate.
- A significantly greater mass of whole plants and roots was found on the substrate Blumenerde

Examined fertilizer factor:

Seed pre sowing treated with Slavol it was sprung up two days earlier.

For pre sowing treatment with Slavol was noticed the highest percentage of germination and the shortest time to the appearance of the first full leaf

Number of leaves at the moment of transplanting was significantly higher in seedlings treated with WUKSAL.

A significantly greater mass of plants and roots was recorded in the treatment of Poly-Feed MAR

Based on the results for the production of lettuce seedlings in the examined area may be recommended pre sowing seed treatment with Slavol, early sowing (first term), Blumenerde substrate and seedlings fertilization with WUKSAL or Poly- Feed MAR.

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**POLLEN GERMINATION OF SOME POMEGRANATE (*PUNICA GRANATUM*L.)  
VARIETIES GROWN IN MONTENEGRO**

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**Abstract**

The research on pollen germination of three most important pomegranate varieties grown in coastal region of Montenegro and the hinterland is presented in this paper. The trial was carried out during the three consecutive years (2002-2004) on two sweet ('Slatki barski' and 'Šerbetaš'), and one sour ('Dividiš meke kore') pomegranate varieties. Pollen germination was tested on solid 1 % agar medium (Agar Plate method) with two concentrations of sucrose (10 and 15 %). 'Slatki barski' and 'Šerbetaš' varieties showed higher degree of germination at the 15 % sucrose medium (65,96 and 71,27 %) than at the medium with 10 % (42,41 and 39,70 %). The degree of pollen germination was significantly lower in 'Dividiš meke kore', on both sucrose concentrations (16,5 and 22,54 %). Therefore, pollen germination parameter in this variety should be tested before planting in mono-varietal orchards.

**Key words:** pomegranate, *Punica granatum* L., pollen, germination

**Introduction**

Pomegranate (*Punica granatum*L.) is commonly grown as a shrub or small tree that can grow up to 5 m. For successful development requires dry and hot summers, with relatively mild winters, although in the tropics is evergreen (Ozguven & Yilmaz, 2000). It was considered as a symbol of fertility, and often quoted in the Bible and the Koran (Mars, 2000). Origin of pomegranate species is usually connected to the area of former Mesopotamia. Zohary and Hopf (2000), however, mainly due to the richness of diversity of wild pomegranate in the Mediterranean area, the countries such as Albania and Montenegro cited as a possible wider gene center of this species. Most of the authors classify pomegranate into *Punicaceae* family, although in the official databases of gene banks it is classified in the family *Lythraceae*. Recent molecular studies (Stover et al., 2007) confirmed this taxonomic affiliation of pomegranate.

Pomegranate, along with olive and fig trees belongs to the longest cultivated fruit species in this area. There is no precise data on the age of this species in our region, but the area of Bar in seventies of the last century had 90 000 of pomegranate bushes (Plamenac, 1977). The number of bushes decreases in the recent period due to the transformation of agricultural land into urban area. There was no evidence of the annual production of this species in Montenegro. However, the worldwide trend in increasing the number of pomegranate trees is obvious, and it is projected to be soon among the leading ten fruit species (FAO, 2010).

Pomegranate forms two types of flowers: hermaphrodite (complete) which is the only able to bear fruit, and functional male flowers which only contribute to a better pollination. Although it is considered to be a self-pollinated plant, much better fruit set was observed when pollinated with cross-pollination (Josanetal., 1979). The aim of this work was to investigate the germination of pollen grains of the most important pomegranate varieties in the

Montenegrin subtropical zone, with purpose to recognize the yield potential and the possibility of establishing mono-varietal plantations.

### Material and methods

The investigation was conducted on three prevalent pomegranate varieties in Montenegro: ‘Slatki barski’, ‘Dividiš’ and ‘Dividiš meke kore’. Pollen of each of three varieties was collected from three different locations (shown on the map 1): Dobra Voda (DV) and Tomba (MN) in the coastal region, and Balabani near Golubovci (ZP) in Zeta-Bjelopavlici valley. The abbreviations used in the paper for the varieties and the localities are presented in the following table.

Table 1. Abbreviation for pomegranate varieties and sampling localities

Abbrev	Variety - locality	Abbrev	Variety - locality
SBMN	Slatki barski - Tomba	ŠMN	Šerbetaš - Tomba
SBBB	Slatki barski - Dobra Voda	ŠBB	Šerbetaš - Dobra Voda
SBZP	Slatki barski - Balabani	DMK	Dividiš meke kore - Balabani



Map 1. Localities of pomegranate plantations used for pollen sampling

Each of the varieties was presented with 5 trees per locality. All trees were of same age (13 and 14 years) and in the full productive period.

Pollen was collected from the flowers which were kept at room temperature in a bowl with water, up to the moment of anthers rupture. Collected pollen was kept in tubes in desiccators before sowing (Melgarejo et al., 2000). The media for pollen germination contained sucrose at two concentrations, 10 and 15 %, with 1 % agar. Germination was carried out in a hermetically sealed Petri dish at a temperature of 28 °C (dryer Hereaus) in the dark. Observation and reading of germination was done after 12 hours on the light microscope with a magnification 100x and in three visual fields. The degree of germination was calculated as percentage from the ratio of germinated and the total number of pollen grains in the visual field.

Statistical analysis was performed by analysis of variance as a two-factorial experiment: genotype in a given locality (factor A) and years of research (factor B). Comparison of differences of mean was tested by Tukey's test for the significance of 0,05 and 0,01 %.

### Results and discussion

#### Pomegranate pollen germination in 10 % sucrose solution

Pollen germination depends on many internal and external factors. A high percentage of pollen grains germination determined in the laboratory conditions may indicate its ability to be good pollinator (Nini -Todorovi , 1989). Successful pollination and its good fertilizing power directly determine the number of seeds in the fruit, and thus its largeness (Derin & Eti, 2001).

Table 2. –Pollen germination in 10 % of sucrose solution, comparison of differences

Variety (A)	Year (B)			$\bar{X}_A$	HSD <sub>A</sub>
	2002	2003	2004		
S. barski DV	41,77 ab	34,51 b	42,95 ab	39,74 a	HSD <sub>0,05</sub> = 4,6011 HSD <sub>0,01</sub> = 5,5062
S. barski MN	42,46 ab	34,97 b	41,66 ab	39,70 a	
S. barski ZP	39,75 ab	33,93 b	41,34 ab	38,34 a	
Šerbetaš DV	46,33 a	38,39 ab	42,52 ab	42,41 a	
Šerbetaš MN	41,60 ab	35,81 ab	42,95 a	40,12 a	
Dividišmekekore	16,39 c	15,13 c	18,02 c	16,50 b	
	HSD <sub>0,05</sub> = 9,8378		HSD <sub>0,01</sub> = 11,316	F(A)= 0,0000** F(B)= 0,0000** F(AB)= 0,7301 <sup>ns</sup>	
$\bar{X}_B$	38,05 a	32,12 b	38,24 a		
HSD <sub>B</sub>	HSD <sub>0,05</sub> = 2,6598		HSD <sub>0,01</sub> = 3,3496		

Analysis of variance showed that the cultivars statistically significantly differ in the percentage of pollen grains germination in 10 % sucrose solution (table 2). Variation in germination in the years of study has also demonstrated a highly statistically significant difference, while the interaction of these two factors was not significant.

Comparing differences in mean percentage of germinated pollen grains show a statistically significant and significantly lower germination of pollen grains in the year 2003 in all of the tested varieties (32,12 %). Germination in 2002 and 2004 was approximately at the same level (38 %). The lowest pollen germination was in variety 'Dividiš meke kore' (16,5 %), highly significant and significantly lower than in the other tested varieties. Highest germination was in 'Šerbetaš' variety, the average for three years was 41,30 %.

#### Pollen germination in 15 % sucrose solution

Analysis of variance of pollen germination in 15 % sucrose solution showed statistically significant differences between varieties. The year as factor studied also showed statistically significant differences, while the interaction of these factors had no statistical significance.

On the base of the results presented in Table 3, it can be concluded that the lowest pollen germination was in 2003 (53,66 %) in all varieties, and thus highly significant and significantly lower than in the other two years of research.

The highest percentage of germinated pollen grain was registered in 'Šerbetaš' variety from locality Dobra Voda (71,27 %), significantly higher when compared to the varieties 'Slatki barski' and 'Dividiš meke kore' from location Balabani.

Table3 - Pollen germination in 15 % of sucrose solution, comparison of differences

Variety (A)	Year (B)			$\bar{X}_A$	HSD <sub>A</sub>
	2002	2003	2004		
S. barski DV	71,46 ab	58,14 ab	68,27 ab	65,96 ab	HSD <sub>0,05</sub> = 7,316 HSD <sub>0,01</sub> = 8,755
S. barski MN	66,90 ab	59,26 ab	62,53 ab	62,90 ab	
S. barski ZP	62,59 ab	54,47 b	67,50 ab	61,52 b	
Šerbetaš DV	72,67 a	68,12 ab	72,92 a	71,27 a	
Šerbetaš MN	70,90 ab	61,29 ab	68,19 ab	66,79 ab	
Dividišmekekore	27,14 c	20,67 c	19,82 c	22,54 c	
	HSD <sub>0,05</sub> = 15,642		HSD <sub>0,01</sub> = 17,993	F(A)= 0,0000** F(B)= 0,0000** F(AB)= 0,5274 <sup>ns</sup>	
$\bar{X}_B$	61,95 a	53,66 b	59,87 a		
HSD <sub>B</sub>	HSD <sub>0,05</sub> = 4,229		HSD <sub>0,01</sub> = 5,326		

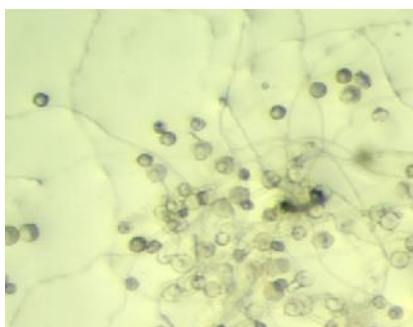


Photo 1 – Pollen germination in 15 % sucrose medium in ‘Šerbetaš’ variety

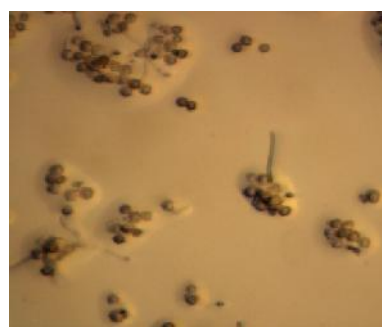


Photo 2 – Pollen germination in 15 % sucrose medium in ‘Dividiš meke kore’ variety

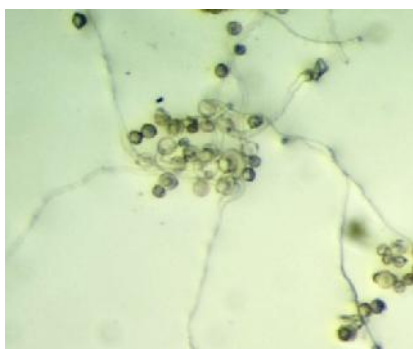


Photo 3 – Pollen germination in 10 % sucrose medium in ‘Šerbetaš’ variety

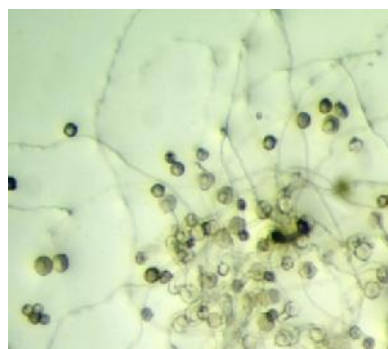


Photo 4 – Pollen germination in 15 % sucrose medium in ‘Slatki barski’ variety

Considering the threshold of significance 95 %, ‘Šerbetaš’ variety from Dobra Voda location had significantly higher percentage of germinated pollen grains in comparison to ‘Slatki barski’ from Tomba site and the other two tested varieties. The lowest germination was detected in ‘Dividiš meke kore’ variety, highly significant and significantly lower when compared to all other varieties (photo 2). The low percentage of pollen grains germination in ‘Dividiš meke kore’ variety is pointing out the possible problems when grown in mono-varietal plantations due to the reduction of fruit set as a consequence of poor fertilization. In three-year study period the lowest germination was in 2003 that may be a consequence of unusually high temperatures during the flowering period. Maximum temperature in May and



June was 30,6 and 35,7 °C in Bar and 32,4 and 38,5 °C in Balabani, which is higher of average temperature for this region.

The difference in the average pollen germination observed in relation to the sucrose concentration was smallest in 'Dividiš meke kore' variety, only 6,04 %. The highest difference in pollen germination related to this factor was recorded in 'Šerbetaš' from Dobra Voda site (28,86 %).

Best germination of pollen grains were registered when the grains were grouped close to each other (Photos 1 and 4), also reported by Pejkić (1998). This phenomenon is considered to be a consequence of greater amounts of secreted enzymatic material and other stimulus, which form "bridges" and connect the pollen grains in the immediate vicinity.

Similar results in examining pollen germination in pomegranate other authors have reported. Džubur (1999) stated that the pollen germination in examined wild pomegranate biotypes ranged from 19,68 % on 10 % sucrose solution (hanging drop), up to 72,98 % in 15 % sucrose solution. Also, the difference in pollen germination among the study years was noted. Wetzstein et al. (2011) while examining pollen germination of pomegranate variety 'Wonderful' in 12 % sucrose solution concluded that the germination depends to a large extent on the temperature conditions. Important is to note that 0,062 % of CaNO<sub>3</sub> and 0,024 % of boric acid was added into the sucrose solution. The highest percentage of pollen germination (74 and 79 %) was registered at 25 and 35 °C, and that there were no significant differences in germination of pollen isolated from anther of hermaphroditic and functionally male flowers. Increase of temperature up to these values increase the pollen germination, while the higher and lower temperatures had negative effects on germination. Pollen grains from both types of flowers had spherical shape and length of ≈ 20µm.

The addition of micro elements, especially B, significantly increased the percentage of pollen germination (Pejkić, 1998). Similar allegations were given by Derin & Eti (2001) while examining the pollen grain germination of 'Hiczas' variety and clone 31 N 06. The authors stated that the production of pollen grains is higher in functionally male flowers, whose function is in better fertilization. Nalawadi et al. (1973) as the best for testing pollen germination suggested the 10 % sucrose medium, while the results of Chitale and Deshpande (1970), showed the highest germination in the medium with 20 % sucrose.

Studying the pollen germination in *Rosa dumalis* and *Rosa villosa* Seazai (2007), by quoting other authors, concluded that pollen germination depends on the environmental conditions as well as on good condition and nutritional status of the plant.

### Conclusion

The highest degree of pollen germination was in 'Šerbetaš' variety from Dobra Voda locality (71.27 %) at 15 % sucrose solution. Pollen germination was high in all the years of research in 'Slatki barski' variety.

'Dividiš meke kore' variety had significantly lower pollen germination in both sucrose solutions and in all three years of research, suggesting the necessity to provide another variety as pollinator to obtain better fertilization and fruit set.

Germination of pollen grains varied considerably, depending on the environmental conditions in some years.

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**SEED GERMINATION AND MORPHOLOGICAL PROPERTIES OF SEEDLING  
GENOTYPES OF CORNELL FROM UPPER POLIMLJE REGION**

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**Abstract**

The results of seed germination and morphological characteristics of 11 seedling genotypes of cornel (*Cornus mas* L.) taken from natural population of Upper Polimlje Region from 2000 through 2004. Cornel is a long-lived plant, but is exceptionally slow at the beginning of growth. Seeds do not germinate in the first year. They germinate afterwards. The seedlings grow very slowly. Cornel starts producing fruit after 8, 9 or 11 years. The seeds of tested genotypes hardly germinated in the second year. The best germination was with the seed of genotype BP 25 – 48 %. Seedlings of cornel grow very slowly, especially in the first year, in the second year their growth is considerably faster. The average height of one-year-old seedlings is 27,54 cm, and two-year-old ones are 80,27 cm. The diameter of two years old seedlings was between 0,5 cm (genotype BP 36) and 1,16 cm (genotype BP 16). The maximum uniformity with respect to the diameter of two-year-old seedlings was found in genotype BP 07 ( $Cv=7,85\%$ ), and in terms of height in genotype BP 16 ( $Cv=9,38\%$ ).

**Key words:** cornel, seed germination, morphology, seedlings

**Introduction**

Wild fruit species present a genetic potential of huge importance for selective breeding. (Koji and Mratini, 1997) Their cultivation would significantly enrich the diversity of cultivated fruit species. Benat and Blaho (2001) claimed that cornel is the tree which grows slowly, but have a long life span, from 100 to 200, even up to 250 years. There are no cornels as cultivars throughout the world, on the other hand, this fruit in natural populations is present in large areas. The cornel (*Cornus mas* L.) was described by the authors who wrote about wild flora and medical plants. They put emphasis on the healing and beneficial effects of teas which are made from almost every part of this plant or from its fruit products. A small number of them paid attention on a cornel as a fruit species which could be cultivated on fruit fields (Janimovi and Božovi, 2003).

The cornel's multiplication can be both generative and vegetative. Generative multiplication is used for getting seedlings for park decorations as well as getting rootstock for their grafting. The aim of this work is getting cornel genotypes from natural population from Upper Polimlje area which could be good as generative rootstocks.

### Materials and methods

The results of seed germinations and morphological properties of seedlings of the 11 cornel (*Cornus mas* L.) genotypes, which were selected from natural population of Upper Polimlje area during 2000 to 2004, have been presented in this work. The seeds were gathered in the time of full technological fruit maturity. The seeds were taken from the fruits and, after being dried, stratified in plastic bags in the fridge, at the temperature of 2 - 4 °C during winter, with the wet control.

Before sowing the seed was soaked in 0,03 % ortocid. Sowing was done in spring, in March-April, with 70 cm distance between rows and 4 cm in rows.

Seed germination has been calculated by determining the ratio between the total number of sowed seeds and the sprout plants, and is presented in percents. The diameter of one-year and two-year seedling is taken just above the root collar.

The collected data were statistically analyzed in SPSS for Windows, version 7,5. Statistical analysis included the analysis of variance and testing the significance of differences among genotypes using Duncan test. The differences in height and diameter of the seedlings is done with Coefficient of variation (CV).

### Results and discussion

The cornel seed germination and seedlings initial growth was after two years. The average seed germination during testing was 31,24 % (Table 1.). The best average seed germination had the genotype BP 25 – 48 % and the worst the genotype BP 07 – 10,66 %.

Table 1. Seed germination in the genotypes of Cornel, Upper Polimlje, 2000-2004

Genotype	2000/2002 (%)	2001/2003 (%)	2002/2004 (%)	Average (%)
BP 01	24,0	36,0	28,0	29,33 <i>abcd</i> *
BP 04	16,0	32,0	32,0	26,66 <i>abc</i>
BP 06	44,0	40,0	36,0	40,00 <i>bcd</i>
BP 07	12,0	12,0	8,0	10,66 <i>a</i>
BP 16	12,0	16,0	40,0	22,66 <i>ab</i>
BP 17	36,0	40,0	56,0	44,00 <i>cd</i>
BP 21	24,0	32,0	36,0	30,66 <i>abcd</i>
BP 22	44,0	20,0	16,0	26,66 <i>abc</i>
BP 25	48,0	56,0	40,0	48,00 <i>d</i>
BP 36	48,0	15,0	40,0	34,33 <i>bcd</i>
BP 40	40,0	32,0	20,0	30,66 <i>abcd</i>
Average	31,63	30,09	32,0	31,24

\*Values marked with different letters are statistically different at the level P=0,005 (Duncan's test)

The genotype BP 07 was put in the first group with the smallest number of germinative seeds and statistically significantly different from the genotypes BP 06, BP 36, BP 17 i BP 25 using Duncan test, whereas genotype BP 25, with the highest number of germinative seeds and statistically significantly different from the genotypes BP 04, BP 16, BP 22 i BP 07.

It is very hard for cornel seed to germinate in natural conditions and it usually happens in the second year. For the seeds to germinate, a period of delayed ripening has to pass, the so called

jarovization, which is one of the reasons why cornel is poorly represented in the form of plantations (Meženski, 2005). The period of ripening is between 20 and 200 days and depends on the species and cultivars (Ognjanov, 1991), and according to Vujanic-Varga (1987) sometimes cornel seed to a full 29 months. Our results show that the nonstratified seed, sown in autumn, sprouts no earlier than in spring of the second year. The autumn sowing of mature seed is also called the „dead” one because in the following vegetative period the result is not seen. The cornel seed, as a rule, germinates and grows around a year or a year and a half. This is the consequence of hard cornel seed. The hard endocarp is the obstacle for germination. Many researchers have studied the possibility of accelerating germination of seeds so that they act on the endocarp mechanical, physical or chemical scarification. But none of those ways proved to be reliable (Dudukal et al., 1990).

The average height of one-year old seedlings was 27,54 cm and the diameter 0,26 cm (Table 2). The smallest height of two-year old seedlings was 64,66 cm at genotypes BP 40. And the biggest 97,33 cm at genotype BP 22 (Table 3). The average height of all two-year-old seedlings of every genotypes was 80,27 cm. The diameter differed from 0,5 cm (genotype BP 25) to 1,15 cm (genotype BP 07). The maximum uniformity in diameter of the two year old seedlings was seen at the genotype BP 07 ( $Cv=7,85\%$ ), and in height at the genotype BP 16 ( $Cv=9,38\%$ ).

Table 2. Morphological properties of one year seedlings of Cornel, 2002-2004

Genotype	2002		2003		2004		Average 2002-2004.			
	heigh (cm)	diametar (cm)	heigh (cm)	diametar (cm)	heigh (cm)	diametar (cm)	heigh (cm)	CV (%)	heigh (cm)	CV (%)
BP 01	38,59	0,41	31,33	0,24	32,56	0,31	34,16 c	11,01	0,32 c	36,28
BP 04	33,07	0,21	24	0,2	25,43	0,18	27,50 abc	15,38	0,19 a	7,43
BP 06	32,52	0,27	23,33	0,3	25,63	0,27	27,16 abc	24,57	0,28 abc	26,51
BP 07	28,7	0,19	21	0,25	22,78	0,2	24,16 a	25,94	0,21 abc	21,39
BP 16	28,19	0,22	21,66	0,3	23,65	0,24	24,50 a	27,04	0,25 abc	23,94
BP 17	24,08	0,34	29,33	0,29	23,57	0,3	25,66 ab	28,17	0,31 bc	18,01
BP 18	30,83	0,29	37	0,28	30,66	0,27	32,83 bc	21,57	0,28 abc	13,73
BP 22	23,05	0,21	28,33	0,27	23,11	0,22	24,83 ab	19,63	0,23 abc	25,06
BP 25	31,09	0,2	23,66	0,22	25,23	0,2	26,66 abc	23,91	0,21 a	8,01
BP 36	24,45	0,21	31,66	0,22	25,88	0,2	27,33 abc	24,68	0,21 ab	5,62
BP 40	28,02	0,22	29,33	0,42	27,13	0,3	28,16 abc	23,57	0,31 c	59,99
Average	29,32	0,25	27,33	0,27	25,77	0,25	27,54	23,45	0,26	33,56

In the first year of growing, according to SloviC (1960), the fastest growing ones are, for example, peaches (92,57 cm) as short-lived fruit species, a bit slower are long-lived fruit species, such as wild pear (32,33 cm), walnut is slower (17,02 cm) and the slowest are wild forest trees – Macedonian oak (11,81 cm) and black pin (1,99 cm). This was also true in the case of cornel seedlings in the first year. As a result of this the grafting of two-year old seedling was shown in the paper of Bijelic at al. (2013). The average height of two-year old cornel seedlings was 80,27 cm, and that means that they need two years more to be like peaches.

Table 3. Morphological properties of two year seedlings of Cornel, 2000-2004

Genotype	2003		2004		Average			
	heigh (cm)	diametar (cm)	heigh (cm)	diametar (cm)	heigh (cm)	CV (%)	diametar (cm)	CV (%)
BP 01	95,9	1,1	89,42	1	92,66 a	18,35	1,05 bc	10,96
BP 04	79,16	0,8	82,16	0,75	80,66 a	35,93	0,78 abc	38,43
BP 06	76,46	0,81	75,54	0,79	76,00 a	39,93	0,80 abc	67,29
BP 07	73,43	1,17	67,89	1,13	70,66 a	20,47	1,15 c	7,85
BP 16	87,95	1,15	94,05	1,16	91,00 a	9,38	1,16 c	46,57
BP 17	89,95	0,69	90,71	0,71	90,33 a	14,06	0,70 abc	11,19
BP 18	77,66	0,93	84,34	0,96	81,00 a	25,05	0,95 abc	39,25
BP 22	96,51	0,82	98,15	0,84	97,33 a	16,73	0,83 abc	59,59
BP 25	61,66	0,68	75,00	0,71	68,33 a	25,69	0,70 abc	17,32
BP 36	70,18	0,50	70,48	0,52	70,33 a	9,67	0,51 a	20,4
BP 40	61,12	0,62	68,20	0,65	64,66 a	28,94	0,64 ab	30,01
Average	79,09	0,84	81,44	0,83	80,27	23,64	0,84	38,79

The seedling growth is the result of all internal and external factors. The internal ones are in genetic basis. Using seeds obtained very variable seedlings with little similarity. By sowing seeds of large cornel fruit forms obtained offspring that shows great variability (Meženski, 2005).

Heterozygose hereditary basis influences the variability and results with different quality of the material obtained. In order to preserve all the varietal characteristics their multiplication should necessarily be vegetative.

### Conclusion

The cornel is a long-lived fruit, but with extremely slow growth and development in the first years. The seeds do not germinate in the first year, they start growing the next year, and seedlings grow slowly. Therefore, it is necessary to produce seedlings, to which best selectios from this region shoul be grafted, which would ensure first fruits in the second or third year after grafting. The examined seed genotypes germinated in the second year, and the best one was BP 25. During the first months they need to be in the shadow. The cornel seedlings are slow in growth, so grafting can be done on two-year or three-year old seedlings

The average height of one-year-old seedlings was 27,54 cm, and two-year-old ones 80,27 cm, which means that they need two more years to grow like peaches. The diameter of two-year-old seedlings was between 0,5 cm (genotype BP 25) and 1,16 cm (genotype BP 16). The maximum uniformity with respect to the diameter of two-year-old seedlings was found in genotype BP 07 ( $C_v=7,85\%$ ), and in terms of height in genotype BP 16 ( $C_v=9,38\%$ ).

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**METHODOLOGY FOR ACIDITY CORRECTION OF DEFICIENT MUSTS BASED ON GRAPE MATURATION INDICES AS PART OF PRECISION OENOLOGY**

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**Abstract**

Acidity corrections are one of the most important pre-fermentative operations, with significant consequences on wine quality. The proposed methodology is based on the study of two grape maturation indices, °Brix/%TA and °Brix x pH<sup>2</sup>, which were determined and evaluated before and after the application of acidity correction. This paper proposes a methodology in agreement with the principles of precision oenology for acidity corrections of deficient musts, especially those from areas located in European zones CII.

**Keywords:** precision oenology, maturation indices, quality assurance, acidity corrections

**Introduction**

Harvesting of grapes is one of the most critical decisions of oenologists with influences on the quality of the future wine. The best moment to harvest may be determined using various physico-chemical parameters such as Brix degrees, total titratable acidity (TA), pH and, last but not least, sensory analysis.

Oenologists may encounter two difficult situations during the harvest period: the first one when the grapes are underripen (high acidity, low sugar level, "green" flavors and harsh tannins) and the second one when the grapes are overripen (low acidity, high sugar level and off- or uncharacteristic flavours) (Zoecklein *et al.*, 1999). When the second situation occurs and some times even in the case of normally ripen grapes, there is a need for acidity corrections. The acidity correction is always advisable due to the positive effect of TA and pH on solubility of tartrates and proteins (Boulton *et al.*, 1998), on color stability (Brise Maria Josephine, 2007), on selectivity of microorganisms during fermentation and aging (Guzzo *et al.*, 2009), on oxidation-reduction reactions (Zoecklein *et al.*, 1999), on production of fine flavours (Boulton *et al.*, 1998) and finally, but most importantly, on the taste balance (Zoecklein *et al.*, 1999) that has to be in concordance with the desired wine style intended by the oenologist and desired by the consumers.

Taste balance of grapes at harvest time can be quantified by some maturation indices such as °Brix/%TA and °Brix x pH<sup>2</sup>, which aim to show the optimal equilibrium between sugar and acidity in the berry, which will lead in the end to an optimum balance of ethanol and acidity in wine (Zoecklein *et al.*, 1999). The application of °Brix/%TA index was suggested by Amerine and Winkler (1940), and subsequently, the introduction of °Brix x pH<sup>2</sup> was suggested by Coombe *et al.* (1980) pointing out the faults of °Brix/%TA index, caused by the lack of reliability of total titratable acidity as measurable parameter. The titratable acidity does not necessarily measures the total acidity, while the pH is a much reliable parameter.

•**Brix/%TA index** (Amerine, M. *et al.*, 1940; Gallander, 1983) was evaluated by various authors and it was suggested that it should range between 30 and 32 (Gallander.,



1983), 30 to 35 (Cox, 1999) or 37 to 38 in the case of overripen grapes (Amerine *et al.*, 1970; Amerine *et al.*, 1980).

•**Brix x pH<sup>2</sup> index** (Coombe *et al.*, 1980; Amerine *et al.*, 1970) was evaluated by various authors and it was suggested that it should be around 220 for white wines and 260 for reds (Cox, 1999). Overripen grapes may reach values over 260 for this index (value of 311 in case of 24 °Brix and 3.6 for pH, for example), which places them beyond the optimum values which usually indicate taste balance. In these cases the oenologists should take into consideration the acidification of the resulted must.

By reviewing several works (Amerine *et al.*, 1940; Amerine *et al.*, 1970; Amerine *et al.*, 1980; Gallander, 1983; Cox, 1999), we derived the following normal range values and optimal values for these indices (Table 1).

Table 1. Normal range and optimal values of maturity indices of grapes for the production of balanced wines

Index	Normal range values		Optimal values	
	White grapes	Black grapes	White grapes	Black grapes
•Brix/%TA	27-32	32-35	28	33
•Brix x pH <sup>2</sup>	200-240	245-280	220	260

It should be mentioned that some black grape cultivars grown in warm climates usually achieve these optimal values before reaching full phenolic maturity or development of flavor compounds and for this reason this methodology has its limitations. As mentioned before, °Brix x pH<sup>2</sup> index may have values in excess of 260, as, for example, 350 in the case of a red variety grown in a warm climate (Bisson, 2001).

## Materials and methods

**Raw materials:** The maturity indices were analyzed for five different grape varieties harvested in a vineyard located in the south of Romania, classified as a CII European zone, where the climate conditions determine a sharp drop in acidity during the normal maturation period. The indices were calculated in the must, before and after acidification with various amounts of tartaric acid, done to adjust these indices and bring them inside the desirable ranges. Grape varieties analyzed were: Crâmpoie (ID 1, 2, 3), Sauvignon blanc (ID 4), Royal Feteasca (ID 5), Italian Riesling (ID 6) and Merlot (ID 7). The first six varieties are white and the last one red.

**Methods of analysis and equipments:** Brix degree was determined with a portable ATC Brix refractometer and the pH with an Ino Lab pH 720 (AOAC, 960.19). Total titratable acidity (TA) was determined with TitroLine easy Schott Instruments until the end point of titration at pH 8.2 was reached (AOAC, 962.12), while the buffer capacity ( ) was determined with the same equipment by titration with HCl 0.1 N until 1 pH unit was dropped. The alkalinity of the ash (AA) was calculated based on titratable acidity and buffer capacity, in accordance to the mathematical relations presented hereafter.

**Calculations:** In order to achieve practical goals, it will be considered that a single monoprotic acid HV is present in the must. For this case acid dissociation constant can be easily calculated based on the laboratory determinations of pH, total titratable acidity (TA) (Moreno *et al.*, 2012). Thus, the equilibrium reaction can be represented as:  $HV \leftrightarrow H^+ + V^-$ .

As shown in this chemical equilibrium, the acids present in musts are partly dissociated. The anions formed in this reaction are neutralized by cations [M<sup>+</sup>] from the must

leading to electrochemical neutrality. Thus, the following relations, can be established (Moreno J. *et al.*, 2012):  $[V^-] = [M^+] = AA$  and  $[HV] = TA$ , where:  $[V^-]$  - anions from musts;  $[M^+]$  - cations from musts (alkali metals);  $AA$  - ash alkalinity;  $[HV]$  - undissociated acid from must;  $TA$  - total titratable acidity of the must;

In accordance to the Mass Action Law and Henderson Hasselbalch equation (ârdea, 2007; Usseglio Tomasset 1992; Moreno J. *et al.*, 2012), the value of the acid dissociation constant ( $K_v$ ) of the above equilibrium can be calculated as:

$$K_v = \frac{[V^-] \times [H^+]}{[HV]}, \text{ where: } pK_v = pH - \log_{10} \frac{[V^-]}{[HV]} = pH - \log_{10} \frac{[AA]}{[TA]}$$

Because the determination of the alkalinity of the ash (AA) is very laborious, it can be indirectly calculated by taking into account the buffer capacity and applying the following equation (ârdea C., 2007; Usseglio Tomasset 1992; Moreno J. *et al.*, 2012):

$$\beta = \frac{L}{\Delta pH} = \ln(10) \times \frac{TA \times AA}{TA + AA}, \text{ where: } AA = \frac{\beta \times TA}{\ln(10) \times TA - \beta}, \text{ where:}$$

$\Delta pH = pH_i - pH_f$  ;  $pH_i$  - initial pH value of must;  $pH_f$  - final pH value of must;  $L$  - titre of HCl 0.1 N, in ml;  $\ln(10)$  - natural logarithm of 10 = 2.3026;  $TA$  - total titratable acidity in meq/l, determined by physicochemical analysis;  $AA$  - alkalinity of ash in meq/l, calculated indirectly from physicochemical analysis of ;

According to the Henderson Hasselbalch equation the  $pK_v$  value for hypothetical monoprotic acid in musts (equivalent to the combination of each acid present) and the pH value can be calculated (Moreno J. *et al.*, 2012):

$$pK_v = pH - \log_{10} \frac{[AA]}{[TA]}, \text{ where: } pH = pK_v + \log_{10} \frac{[AA]}{[TA]}$$

After the addition of tartaric acid, precipitation phenomena of potassium bitartrate will occur, due to the abundance of potassium and low saturation point of this salt in musts. Also, salt precipitation has an effect on the total titratable acidity (TA) and the alkalinity of ash (AA). Thus, the measurement or calculation of TA and pH after acidification is also necessary. In order to calculate them we go through two stages (Moreno *et al.*, 2012):

**Stage I.** Effects of acidification on AA and TA can be calculated using the following relations (Moreno J. *et al.*, 2012):  $Stage\ I\ AA = AA - H_2T + H_2T$  and  $Stage\ I\ TA = TA + 2 \times H_2T$

**Stage II.** Effects of bitartrate precipitation on AA and TA can be calculated using the following relations (Moreno J. *et al.*, 2012):  $Stage\ II\ AA = Stage\ I\ AA - H_2T$  and  $Stage\ II\ TA = Stage\ I\ TA - H_2T$ , where:  $H_2T$  - meq/l of tartaric acid added.

## Results and discussion

For our grapes harvested in a vineyard affected by a hotter than usual climate, located in a CII viticultural zone, a series of physico-chemical analyses were performed to the resulted musts, prior to their acidification. The results are shown in Table 2.

As it can be seen in Table 2, maturity indices of analyzed musts have much higher values than the optimal ones from Table 1. This is due to the obtainment of musts from grapes with very

low acidity and normal to medium-high levels of sugar, specific for the vineyard where we conducted the study. These musts need an acidification in order to achieve a good taste balance between sugar and acid which will finally have an impact on the ethanol/acidity balance of wine.

Table 2. Quality parameters and maturity indices determined in musts prior to acidification

ID	Grape colour	*Brix	pH	TA, %	, meq/l	AA, meq/l	pKv	*Brix/%TA	*Brix x pH <sup>2</sup>
1	white	21.6	3.51	0.56	72.03	53.85	3.652	38.57	266.11
2	white	22.4	3.54	0.55	72.26	54.88	3.666	40.73	280.71
3	white	22.8	3.59	0.54	71.01	53.97	3.715	42.22	293.85
4	white	24.4	3.85	0.47	80.88	79.99	3.744	51.91	361.67
5	white	21.8	4.12	0.38	70.00	76.07	3.943	57.37	370.04
6	white	23.6	3.53	0.55	75.19	58.89	3.625	42.91	294.08
7	black	23.0	3.99	0.42	67.15	60.89	3.953	54.76	366.16

Due to the high drop in grape acidity before harvest, the quantities of tartaric acid needed to be added for correction in musts are sometimes even higher than the maximum allowed by the legislation, i.e. 1.5 g/l tartaric acid for European CII zones (Order 645, 2005). The addition of very large amounts of tartaric acid prior to fermentation is not only limited by law, but it should be avoided also because of the negative effect of this acid on the flavor of the wine. In this particular case, oenologists should also rely on sensory analysis when they acidify these kinds of musts.

In order to bring the maturity indices closer to the optimum values presented in Table 1, the musts were acidified with certain amounts of tartaric acid (Table 3), decided by a panel of 3 oenologists in accordance to the best sensory taste balance achieved in laboratory samples corrected with various levels of acidity. The results of the physicochemical analyses performed after acidification are shown in Table 3.

Table 3. Quality parameters and maturity indices determined in musts after acidification

ID	Grape colour	Amount of tartaric acid used (g/l)	Brix, %	pH	TA, %	*Brix/%TA	*Brix x pH <sup>2</sup>
1	white	2	21.6	3.12	0.69	31.30	210.26
2	white	2	22.4	3.13	0.71	31.75	219.45
3	white	2	22.8	3.15	0.69	32.85	226.23
4	white	3.5	24.4	3.34	0.74	32.98	272.20
5	white	4	21.8	3.35	0.77	28.31	244.65
6	white	4	23.6	3.30	0.67	35.38	257.00
7	black	1.75	23.0	3.68	0.59	38.70	311.48

As it can be seen, the values obtained for the maturity indices after acidity correction are much more close to the normal range of values for these type of wines. However, in order for these musts to be corrected satisfactorily, in all the cases the legal limit of acid addition had to be exceeded. For these situations, in practice, even though the sugar accumulation might not be optimal, an early harvest should be considered.

In order to propose a methodology to adjust the maturity indices by acidification without having to make other physico-chemical determinations after the correction, for the added acid concentrations used in Table 2 we calculated the parameters pH, TA, , AA and maturity indices. The calculated parameters are called „predicted quality indicators” and are shown in Table 4.

Table 4. Predicted quality parameters and maturity indices of musts after acidification

ID	Grape colour	Amount of tartaric acid (g/l)	, meq/l	AA, meq/l	pH	TA, %	°Brix/%TA	°Brix x pH <sup>2</sup>
1	white	2	49.38	27.20	3.08	0.76	28.42	204.91
2	white	2	50.68	28.22	3.12	0.75	29.87	218.05
3	white	2	49.26	27.32	3.16	0.74	30.81	227.67
4	white	3.5	58.84	33.35	3.23	0.82	29.76	254.56
5	white	4	43.01	22.77	3.28	0.78	27.95	234.53
6	white	4	56.13	32.24	3.13	0.75	31.47	231.21
7	black	1.75	58.70	37.57	3.63	0.60	38.66	303.07

Comparison of results (Fig. 1 and 2) were performed for the determined maturity indices against the predicted maturity indices by using correlation matrices of the software package Statistica 10.0. As it can be seen, the correlation coefficients in both cases are very high, and we can conclude that, for the adjustment of the maturity indices through acidity correction we only need to determine the initial pH, titratable acidity (TA) and the buffer capacity ( ) and calculate for the intended amount of acid to be used the predicted maturity indices. The amount of acid to be used will be decided by the oenologist when the predicted maturity indices are satisfactory as compared to the normal range and optimum values and must also not exceed the legal limits.

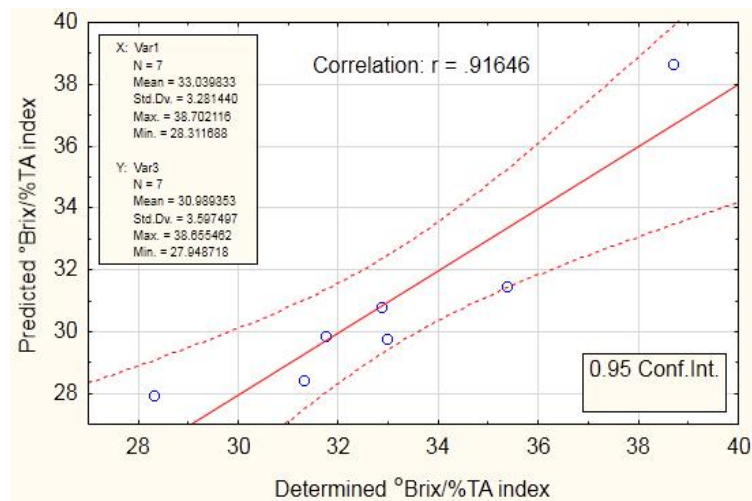
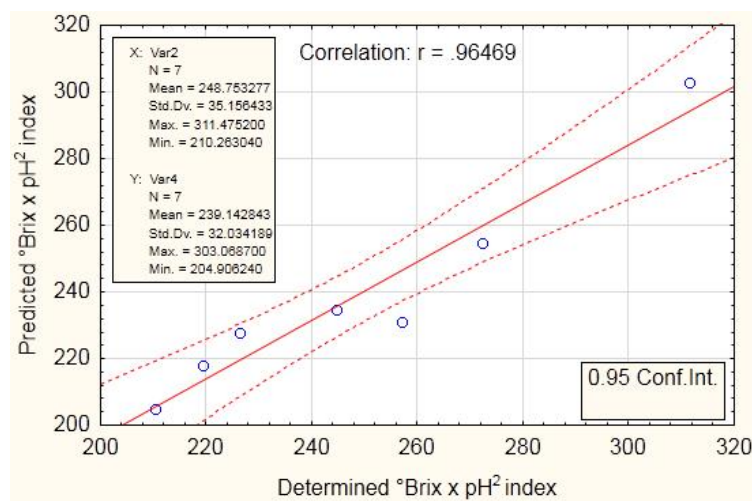


Fig. 1. Correlation between determined °Brix/%TA index against its predicted values


 Fig. 2. Correlation between determined °Brix x pH<sup>2</sup> index against its predicted values

Modeling °Brix/%TA and °Brix x pH<sup>2</sup> indices through acidification with tartaric acid seems to be a good compromise to achieve the taste balance between sugar and acid and finally between ethanol and acid in wines, especially in European CII zones where the aromatic and phenolic ripeness is achieved when the maturity indices are out of ranges, due to the imbalance of °Brix with TA and pH.

It is well known that the acidification of musts is beneficial in case of low TA and high pH, providing a better stabilization of salts and proteins in wines, more color extraction and stabilization for red wines and increased effect of sulfur dioxide on microorganisms during all stages of winemaking process. Moreover, the most important effect of acidity correction is to be encountered in the wine taste balance.

In hot climates, relatively high sugar musts come along with such a lack in acidity, that the maturity indices cannot be brought into the desired normal ranges, irrespective of the acidity corrections. In those cases, the grapes should be harvested before this happens, so that the high alcohol content resulted from all that sugar should not seem imbalanced in a wine with acidity corrected only as much as the legislation permitted and not as much as needed from a sensorial viewpoint. Moreover, with late harvest grapes, over 26 °Brix, it is even harder to find a good compromise, especially as such musts normally do not ferment completely, and the remaining sugar will also have a sensory impact on the final wine, along with the high alcoholic content and low acidity. Conversely, addition of high amounts of acid for correction make some wines seem tart and harsh, while impairing their normal aging, even though some of the apparent harshness decreases in time due to the production of ethyl acid tartrate (Edwards *et al.*, 1985).

### Conclusion

Often, as also acknowledged by the legislation, in European CII zones the acidification of musts is an indispensable operation. To simplify the work of the oenologists, the application the °Brix/%TA and °Brix x pH<sup>2</sup> indices and their adjustment to desired values by addition of tartaric acid appears to be a good and rapid option. By using several theoretical acid correction values, the oenologist can calculate the predicted indices based only on the determinations of the initial pH, titratable acidity (TA) and the buffer capacity ( ). By taking into account the predicted values and the the legal limits, the oenologist can select the most suitable amount of acid to be added in the must for correction. By making these calculations during the maturation period of grapes, the optimum harvest period can also be determined, by pushing the harvest day as far as possible for the achievement of phenolic maturity, but taking also into account the limitations of the acidity corrections.

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## THE INFLUENCE OF THE WAVELENGTH OF LIGHT ON SEEDLINGS LETTUCE GROWING

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### Abstract

The study was conducted at the Faculty of Horticulture in Bucharest, Romania Department of Hortivicol Bioengineering Systems in 2012.

The seedlings production involves high costs for thermal energy. Using LED lighting leads to a shorter growing season and finally to an overall reduction in production costs.

The experiments were performed in growth chamber under controlled conditions regarding temperature, humidity and light. As biological material we used lettuce (*Lactuca sativa* L).

We found that there were differences in the growth of young plants of lettuce. Depending on the combination of colours (proportion between red and blue light) the reaction of plants was differently. All data obtained were processed with National Instruments Vision Assistant software 2009.

The aim of the experience was lettuce growers recommend using LED lighting particularly effective in producing seedlings.

**Key words:** lettuce, LED, growing

### Introduction

Researchers have sought to address expansion space for growing different vegetable in restricted spaces using artificial lighting with LEDs (*Light-emitting diodes*).

At present, farmers are faced with price increases to provide additional lighting in greenhouses, at certain times when the light is low. Using incandescent lamps but causes high power consumption compared to LED lighting. Advantages of using LEDs provide: low-power, high productions, shortening the lettuce crop cycle, uniform plant development, good quality, low water consumption, reducing greenhouse emissions.

LEDs have started to be used in space by NASA since 1960, currently being widely used due to very low energy costs. By the 1980s, the Japanese have applied technology to increase planting, flowers and herbs.

LEDs not only a trend but has become an essential tool for survival in today's competitive market as its application is more diverse. (PARUS [www.parus.co.kr](http://www.parus.co.kr), Plants grow with led lighting).

Light is a form of solar radiation energy that directly or indirectly triggers all living systems. The radiant energy must understand the full range of wavelengths that make solar photosphere radiation reaching the earth's surface. The main source of caloric energy is the radiant energy emitted by the sun. The effect of sun light on vegetable plants grown in protected areas is known. Light is a contributory factor in the growth of vegetable species representing photosynthesis energy support, the biomass structure and training. Light can condition the morphogenesis and organogenesis processes of plant thru intensity, duration and spectral composition.

Light quality directly influences physiological and chemical processes in plants the light spectrum radiation is selectively absorbed by chlorophyll pigments in a wider range than the human eye. Electromagnetic radiation differs in wavelength, colour and energy content. Sunlight spectrum ranges from red, orange, yellow, green, blue to violet, being visible spectrum of sunlight, plus at both ends of the spectrum, invisible to our eye colour, so that at one end is infrared and ultraviolet to the other. Visible light radiation components are absorbed selectively. Chlorophyll absorbs light energy between 250-750 millimicrons, receptive maximum being between 280-500 and 650-680 millimicrons (Morrow, R.C., 2008). It is known that leaves exposed to light predominates chlorophyll. A that better capitalizing red, orange and yellow radiation, but the leaves plants exposed at shade prevailing chlorophyll B and yellow pigments which capitalize blue-violet radiation, which leads to differences in the synthesis of carbohydrates and proteins (Niculita Petru, 2009).

Blue light is important for phototropism (Blaauw and Blaauw-Jansen, 1970 cited by Maticoc, 2012), for stomata opening (Schwartz and Zeiger, 1984, cited by Maticoc, 2012), and for inhibiting seedling growth on emergence of seedlings from a growth medium.

In addition it seems that LED's light improve the pollination and bumblebees activity and has an insecticide effect on aphidiae.

Knowledge the response to light quality vegetable plant allows growers to influence one or the other side of growth and development processes.

By using the proper combination of illumination light, it is possible to increase plant yield by more than 20 percent, while also improving product quality and extend the season of production. (Stalions Drake, 2011).

### **Materials and methods**

The researches were conducted into Department of Hortivicol Systems Bioengineering, Faculty of Horticulture, Bucharest, during 2011-2012.

As biologic material were used two lettuce varieties, Attraction and Murai Rijk Zwaan.

The seeding was carried out in the perlite substrate. The experiments were performed in growth chamber under environmental controlled conditions. It had been provided constant temperature of 22°C during the day for 16 hours and 18°C during 8 hours for night. It was used for each variety 50 seeds in three repetitions. Thus for every variety were sown 150 seeds how many.

The illumination was achieved using white light of neon as a witness and LED light consists of 70% red, 20% blue and 10% green light.

Bioleafez in concentration of 0.5% as fertilizer was used at intervals of 2 days.

In this study the behaviour of lettuce in different light was followed by assessing the percentage of germinated seeds, seedlings height growth dynamics, the dynamics of leaf mass and seedling root system development.

### **Results and discussions**

By counting every day it was determined the percentage of seed germination. Based on observations made we found that the percentage of germination of lettuce seeds was between 97% and 100% for Murai Rijk Zwaan variety and 82% respectively 95% for the variety Attraction. The data presented in Table 1 show that for both varieties germination was higher when using the LED light.



Table 1. The influence of the nature of light on lettuce seed germination

Light	Date of sowing,	Date of sprouting	Percentage of germination, %	
			Murai Rijk Zwaan	Attraction
Neon	10.10.2012	13.10.2012	97	82
LED	10.10.2012	11.10.2012	100	95

The results are amazing because as we can see in the LED light the seeds germinated almost all for both varieties after one single day. In neon light seeds needed 3 days to germinate. Observations on the dynamics of formation of lettuce leaves Attraction and Murai Rijk Zwaan varieties showed that in both varieties formed a greater number of leaves when seedlings growth under LED lighting (Figures 1 and 2).

As we can see from these figures, in both cases the number of leaves formed is higher in case of use LED light. Additionally variety Murai Rijk Zwaan is more productive than the variety Attraction. Thus at every period of 15, 20 and 30 days the number of leaves is higher for Murai Rijk Zwaan compared to variety Attraction.

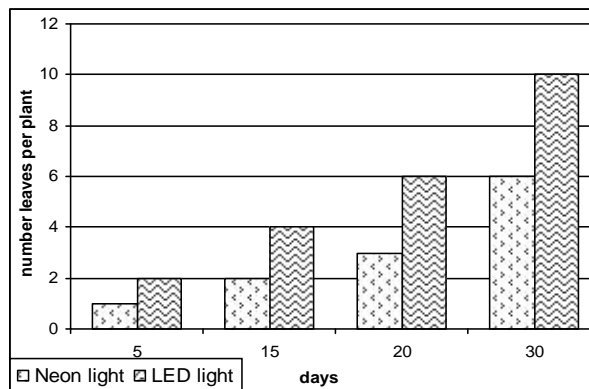
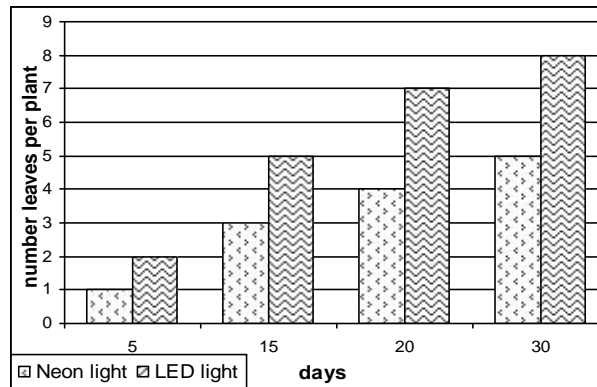


Fig.1 The dynamics of leaves formation at variety Attraction

Fig.2 The dynamics of leaves formation at variety Murai Rijk Zwaan

There was a highly significant correlation of the number of leaf formation (fig. 3 and 4).

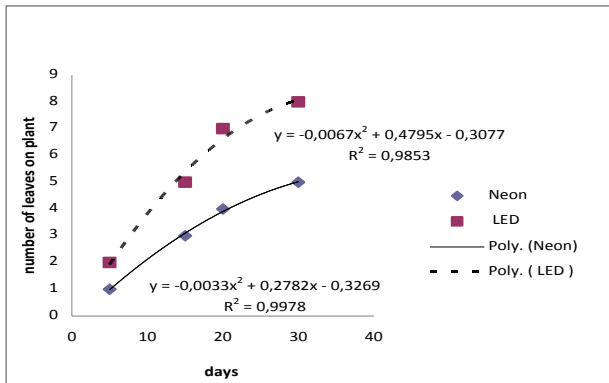


Fig. 3. Dynamics of training leaf at Attraction variety

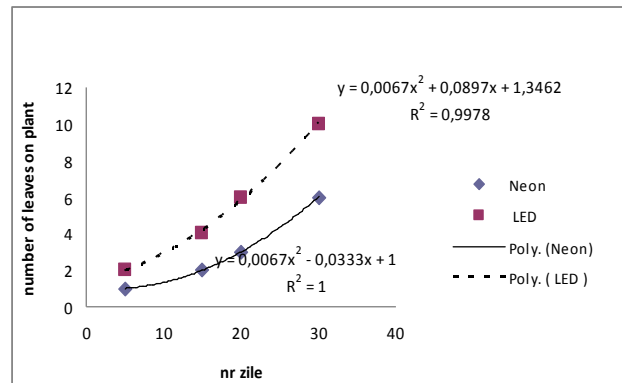


Fig. 4. Dynamics of training leaf at Murai Rijk Zwaan variety

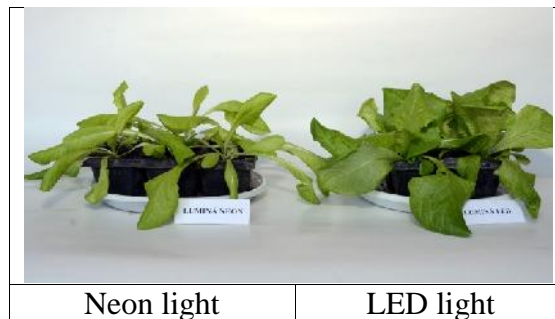


Fig. 5 The appearance in the two light sources after 30 days of variety Attraction.

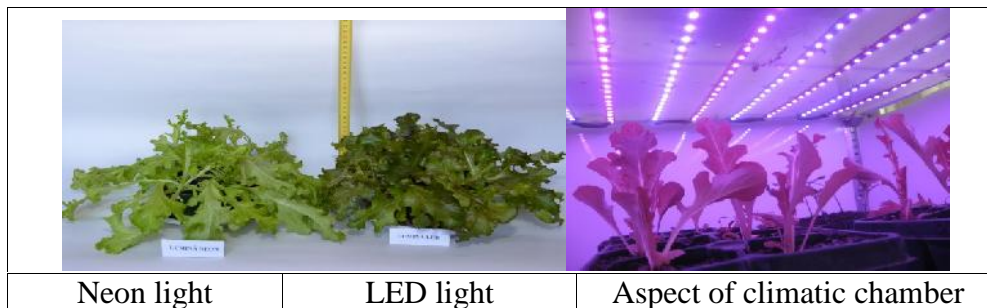


Fig. 6 The appearance in the two light sources after 30 days of variety Murai Rijk Zwaan

After a period of 30 days, the plants (transplants) of lettuce were weighed and dry mater analysed.

Mean mass per plant for both cultivars were higher for LED lighting.

Therefore the variety Attraction had a mean weight with 35.8% higher and the variety *Murai Rijk Zwaan* with 31.8% higher compared to plants exposed to neon light (table 2).

Table 2. The average weight of lettuce plants

Light	Attraction		Murai Rijk Zwaan	
	Mass g/plants	Percent to neon %	Mass g	Percent to neon %
Neon	12.91	100	13.75	100
LED	17.53	135.8	18.12	131.8

Therefore the conditions of cultivation by using LED lighting appear as favouring the development of plants (transplants).

Table 3. The dry matter content of lettuce plants

Variety	Variant	Dry matter %	The percentage of dry matter to V1 %
Murai	V1 - neon	780	100
	V2 -Led	977	125,26
Attraction	V1	741	100
	V2 Led	1069	144,26

### Conclusions

These preliminary experiments show a favourable effect of LED light compared with the use of neon light, light commonly used in control cultures.

For all determinations the results were higher for both varieties in LED light culture conditions.

### Acknowledgements

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This research work was carried out with the support of SC ALTROM SRL, Bucharest, Romania furnished the Led s technology and SC. PROCEMA PERLIT SRL, Romania, furnished the perlite.

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## **SWEET CORN GROWING PERIOD AND MORPHOLOGICAL PROPERTIES IN WET YEAR**

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### **Abstract**

Experiment aims to investigate shortening of sweet corn growing period with application of some technological elements: propagation time, propagation method, floating row cover. The chosen variety was a conventional sweet corn hybrid, very early ripening 'Spirit'. The following growing technologies were compared: 1. Plants transplantation with floating row cover, 2. direct sowing of plants with floating row cover, 3. direct sowing of plants with no row cover (regarded as control).

The transplanted plants had shorter growing period by 13 days, compared to direct sowed covered treatment and were 17 days earlier harvested than control. Interaction of growing technology and plants covering had also favourable effect on some important morphological properties of ears such as weight of husked and unhusked ears, ear length, ear diameter, length of kernel and number of kernels.

**Key words:** earliness, sweet corn, transplantation, fleece covering.

### **Introduction**

Currently, Hungary is not considered as an influential country of the market considering the majority of the vegetables. The only exception is the sweet corn. Although the yield fluctuated, the growing area have grown continuously, in 2002 we gone before French and Hungary became the European leader in sweet corn growing. The impulsive force of great growth, was the canning and freezing industry. Based on its present growing area, the sweet corn is the vegetable which is grown on the greatest area in Hungary and after the sudden and sharp decline in 2003 this plant returned in a rise after 2006. According to the Hungarian Interprofessional Organization for Fruit and Vegetables and Product Board, with a growing area of over 30,000 hectares Hungary is presently the first in the EU. In the case of the former, however, the increase in fresh consumption partly counterbalanced the rate of decrease. In order to promote fresh consumption, as well as to maintain and increase the sweet corn exports, it is necessary to promote investigations able to ensure a further increase in the growing area and yields of sweet corn with the help of the experiences. Of the production technology elements, a number of researchers studied or are currently studying the sowing time of sweet corn. Early sowing is also recommended by Aldrich (1970) for the reason that the roots will penetrate deeper this way, from where they can get water even in periods of drought. The more intensive vegetative growth also takes place during the period of shorter daytime and this way the plants will be smaller and will be less prone to lodge. Several techniques are known in the art for the purpose of early fresh market shipments: seedling growing or direct seeding with temporary plant cover (Kurucz 1998; Hodossi 2004). Direct seeded sweet corn under fleece cover showed earlier ripening and gave better yields in the

experiments of Kassel (1990). The plots under fleece cover reached harvest maturity 12 days earlier as compared to the plots with no cover. Besides, a greater number of missing plants was observed in the plots with no cover. As a result of the greater plant number and the better ear set per plant yields were much higher in the plots with fleece cover. The most widespread method of seedling production is the use of soil blocks (Perczes, 1999) which can also significantly increase earliness. According to the trials of Kurucz (1998) seedling growing advanced harvest by 2 weeks. According to Hodossi (2004) 10 to 12 day earliness can be achieved by planting seedlings grown in soil blocks and 6 to 8 day earliness by seedlings grown in trays. The measurements of Kassel (1990) revealed that the ears of direct seeded corn plants under floating row cover could be harvested 10 days earlier as compared to the plots planted with seedlings and having no cover. The combined application of seedling growing and floating row cover can advance harvest by three weeks as compared to the traditional technology and can give farmers a three to four times greater income (Kurucz, 1998; Perczes, 1999).

### **Materials and methods**

The experiments were set up in 2010 on an area equipped for irrigation at Voivodeni, 10 km SW from town of Reghin, Mures County, Romania. Conventional, reliable and sufficiently known among growers sweet corn variety, Spirit, was used as a reference variety in the variety comparison trials of the Central Agricultural Office. Hybrid has short growing period of 85 days and yellow kernels. Average height of plants is 159 cm, average ear height is 37 cm, ear length 19.6 cm and average ear weight is 245 g. The variety was granted official recognitions in 1988 and has been the dominant variety of the early ripening category till now. In the year prior to the experiment the area was under cabbage.

The following treatments, each with four replications, were applied during the experiment:

P1 = Plants transplantation with floating row cover, (May 9<sup>th</sup> 2010)

P2 = direct sowing of plants with floating row cover (May 9<sup>th</sup> 2010)

P3 = direct sowing of plants with no row cover (May 9<sup>th</sup> 2010) (Control)

For the frame structure of the treatments with cover we used Ø 4.2 mm zinc coated wire coils. The fleece, 60 cm in width, was stretched over a small tunnel of 40 cm in height and then its edges (25-25 cm, respectively) were covered with soil using a hoe and the its ends were tied to the stakes hammered down. The construction of the frame structure and the setting out of the fleece cover were carried out at the same day as direct seeding and out planting.

For the purpose of seedling growing, the seeds were sown on first decade of April 2010, in trays with rigid walls having 7x7 cm size. For growing the seedlings, commercial mix made of white peat 10-20 mm, PG Mix 1 kg/m<sup>3</sup> + micro nutrients, bentonite 40 kg/m<sup>3</sup>, pH 5,5-6,5 was used. The seedlings were planted out at the 3 to 4 leaf phenological stage. At the two propagation times the treatments P1 and P2 were covered with Novagryl floating row cover, having a weight of 19 g/m<sup>2</sup>, (using the small tunnel technique) in order to enhance earliness. The stand was created to contain 60,607 plants per hectare, according to the recommendations of the owner of the variety, at a spacing of 110+40x22 cm in twin rows. Each plot had an area of 6x7m (8 parallel rows and 30 seeds sown in each row). Sowing depth was 3 cm. The edge was the respective outer rows of the 4 twin rows of the plot.

On 24<sup>th</sup> October 2009, 35 t/ha of farmyard manure was worked into the soil with ploughing on area. Nitrogen fertilizer (60 kg/ha) was applied on two occasions, at the 6-7 leaf stage and

at tasseling, in the form of top dressing. The fertilizer application was worked into the soil with a rotary hoe.

Ears were harvested together with the husks, from the two central twin rows. Twenty ears were selected from each row and the following measurements were carried out:

- weight of unhusked and husked ears (g),
- total ear length (cm),
- ear diameter (mm),
- length of kernels (mm),
- number of kernels.

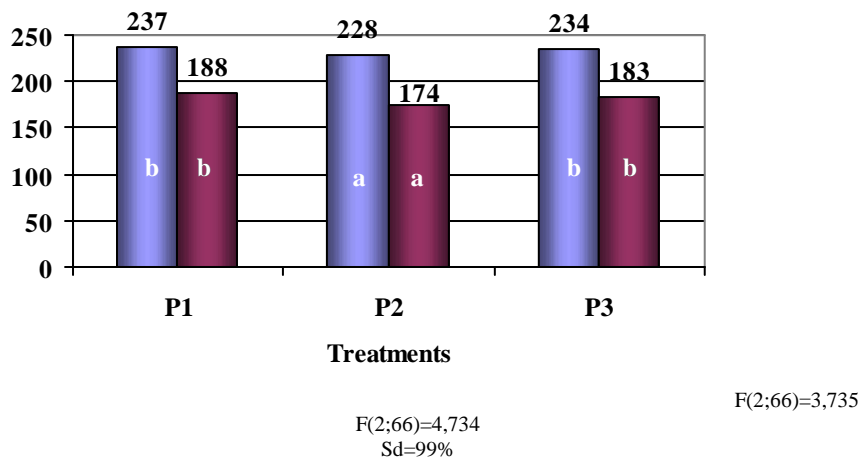
The statistical analysis of the results was carried out by using the programme *RopStat 1.1*. When the standard deviations were identical the mean values were compared by pairs using the *Tukey-Kramer* test, while in the case of the non identical standard deviations the means were compared using the *Games-Howell* test (Vargha, 2007).

*Meteorological conditions.* Under climatic conditions of the Mures County, Romania, the most important requirement for early sweet corn varieties are short growing period and high tolerance to ground frosts in late spring. In wet year 2010, precipitation sum was 85 mm higher from April to July in comparison with multiyear average. Especially wet was period January – March with 75 mm more precipitation than multiyear average, which postponed the direct sowing and out planting from the end of April to beginning of May.

### Results and discussion

According to obtained results, harvesting time (measured in days) was the shortest in the treatments P1 and P2, merely 69, respectively 82 days, i.e. the corns became ready for harvest 17, respectively 13 days earlier than those of P3 (control).

Results of the one of the major characteristics in connection with yield rating, unhusked and husked ear weight, are summarised in Figure 1.

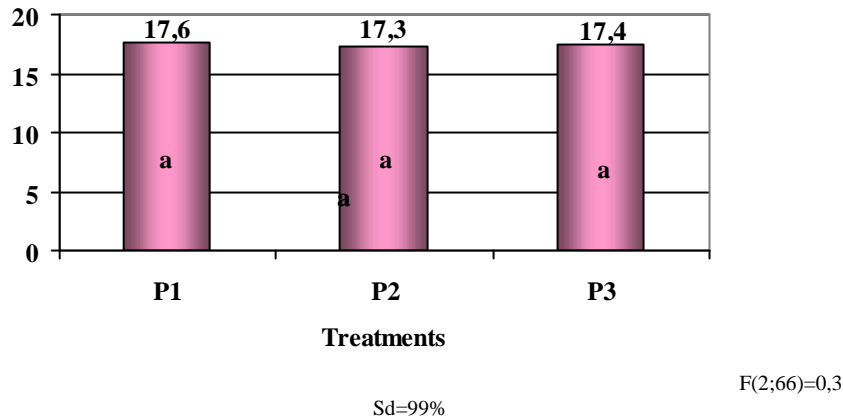


**Fig. 1.** Unhusked and husked ear weight (g).

Analysing the data measured for unhusked ear yield, it is noticeable that the average weight of the ears of the transplanted, covered treatment P1 was significantly (at  $p < 0.01$  level) higher as compared to the sowed, covered treatment P2. Though there was some difference between the plants of the treatments P1 and uncovered, sowed, control treatment P3 in unhusked ear

weight, statistically this was not significant. In case of husked ear weight the same tendencies were observed as in case of unhusked ear weight.

The data concerning, an important characteristics for market appeal (total ear length) are contained in Figure 2.



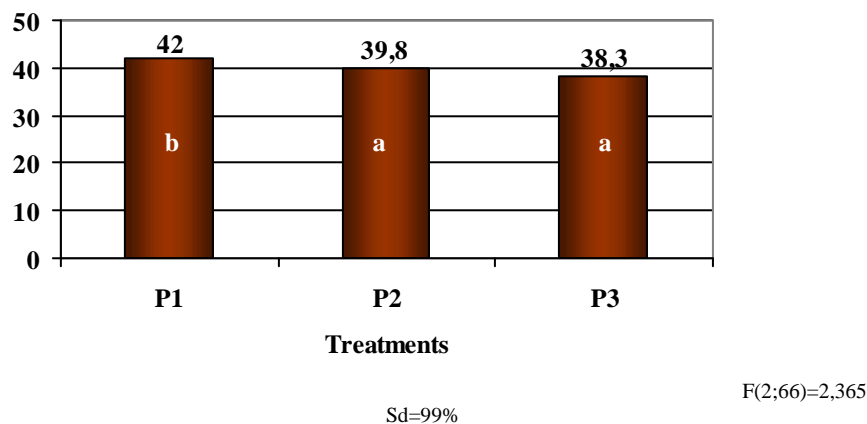
**Fig. 2.** Total ear length (cm).

The length of the covered transplanted treatment P1 was longer compared to the ear length of the uncovered and covered, sowed treatments (P2 and P3).

No statistically demonstrable difference was found between ear lengths of all treatments.

Total ear length not achieved average ear length 19.6 cm as measured in the variety comparison trials.

Other important characteristics for market appeal (total ear diameter), is presented in Figure 3.

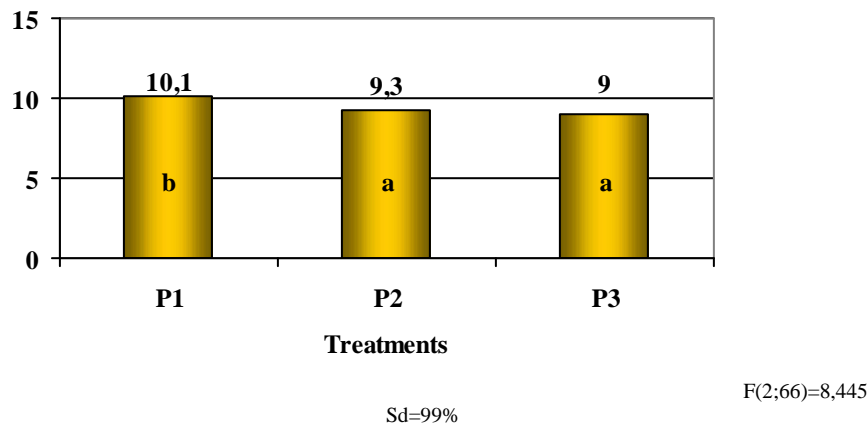


**Fig. 3.** Total ear diameter (mm).

Fleece covering had positive influence on total ear diameter. The measured values were higher in case of covered treatments P1 and P2 compared to uncovered, control treatment P3.

The total ear diameter of transplanted, covered treatment P1 was significantly (at  $p < 0.01$  level) higher as compared to the sowed, covered and uncovered treatments P2 and P3.

Length of seeds is an important characteristic for yield quantity is presented on Figure 4.

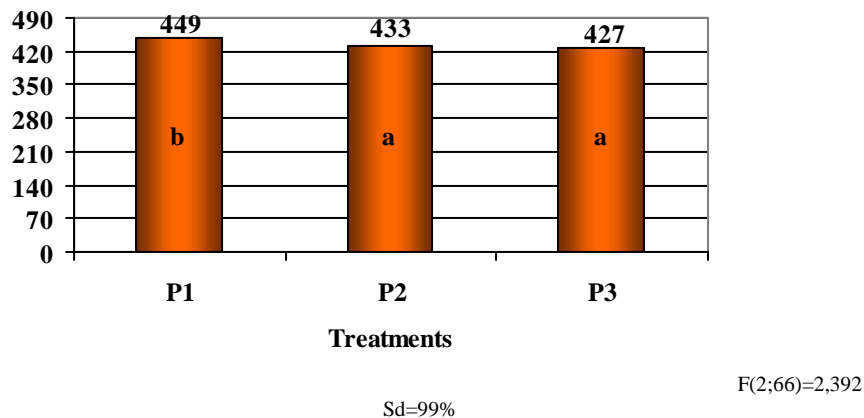


**Fig. 4.** Length of seeds (mm).

Favourable influence of fleece covering is observed in case of seeds length. The measured values were higher in case of covered treatments P1 and P2 compared to uncovered, control treatment P3.

The total ear diameter of transplanted, covered treatment P1 was significantly (at  $p < 0.01$  level) higher as compared to the sowed, covered and uncovered treatments P2 and P3.

Number of seeds is also an important characteristic for yield quantity is presented on Figure 5.



**Fig. 5.** Number of seeds (pieces).

Analysing the data number of seeds, I observed that average number of seeds the transplanted, covered treatment P1 was higher as compared to the sowed, covered and uncovered treatments P2 and P3 (control). In case of this morphological parameter the statistical programme could not demonstrate significantly difference.

### Conclusions

Based on the results of the 2010 year experiment, the following conclusions can be made: The growing period was significantly shortened with transplantation of sweet corn plants compared to direct seeded. Harvest time occurred 17 days earlier in the case of transplantation and floating row cover application compared to direct sowed, uncovered, treatment, and 13 days earlier compared to direct sowed, covered treatment. At the same time the floating row



cover produce 4 days shortening in the growing season between P2 (direct sowing of plants with floating row cover) and P3 (direct sowing of plants with no row cover) treatments.

The fleece covering had favourable effect on studied morphological characteristics of plants that are transplanted and floated with row cover.

In case of direct sowed treatment (P2) the effect of covering had positive effect on total diameter of ears, number of seeds and length of seeds.

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## USE OF VARIOUS YEAST STRAINS TO IMPROVE THE AROMATIC PROFILE OF CHARDONNAY WINES

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### Abstract

Chardonnay is a versatile variety which can lead to various styles of wines. Several yeasts are available in the market for the production of varietal wines, some of them designed especially for the fermentation of this cultivar. However, one of the nowadays trends is to produce new styles of wine, in order to attract new consumers. Chardonnay must from Murfatlar vineyard, 2012 vintage, was fermented with 9 commercially available yeasts, among which some recommended for other grape cultivars than Chardonnay. Control wines obtained based on spontaneous fermentations were also produced. Sensory analysis was performed on the resulted wines, by using the standardized methodology of aromatic profiling with a human panel. In parallel, a dual column GC electronic nose was also used to compare and discriminate among the aromatic profiles of wines. Based on the examination of the aromatic profiles given by the human panel and the volatile profiles discriminations achieved by the electronic nose, it was concluded that some of the yeasts did induce important changes in the wine aroma, but the aromatic characteristics of the cultivar are not completely masked by any of the yeasts used. In accordance, it was possible to obtain a consensus profile of the Chardonnay wines of Murfatlar vineyard, irrespective of the yeast strain used.

**Keywords:** wine, aromatic profile, sensory analysis, electronic nose

### Introduction

In accordance to DNA analysis the Chardonnay cultivar of *Vitis vinifera* ssp. *sativa* resulted from the crossing of Pinot noir and an ancient variety native to Balcan peninsula, Gouais Blanc (Harriet *et al.*, 2009). The first one has its origin directly in a wild vine (*Vitis vinifera* ssp. *sylvestris*) (Jackson, 2009), while Gouais Blanc or Heunisch Weiss, a variety yielding poor quality wine (Hunt *et al.*, 2009), was initially cultivated in Eastern Europe under the name of *Belina Drobna* or *Štajerska Belina* on plots of land considered not suitable for noble vine varieties. During Middle Ages, the Gouais Blanc was brought in Eastern France, where it spread in the same territory where Pinot noir was cultivated, making therefore possible the crossing between the cultivars (Bowers *et al.* 1999). The resulted Chardonnay became, in time, one of the most popular grape varieties in the world.

Although in Romania the surfaces with Chardonnay are under 2000 ha (Antoce *et al.*, 2013), in the world Chardonnay occupies a surface of around 175000 ha (Boubals, 1990) and is made in various style of wines, from still wines to sparkling wines, from fruity to creamy and buttery wines, matured or not in the presence of oak (barrels or alternatives).

In some regions Chardonnay got his fame for a certain style, making the job of selection and wine recognition a bit easier by the consumer, who is able to identify the most typical wines of Chablis, Bourgogne, Argentina, Chile, South Africa, Australia or California made out of this variety. However, in recent times, there is a tendency towards innovation,

including in Chardonnay, especially since some consumers got tired of a certain style, leading in the '90s even to a movement called ABC, “Anything But Chardonnay”, as a reaction mostly towards the oaked wines.

As a result, the modulation of Chardonnay aroma by fermentation with selected yeasts was attempted by many oenologists, while the producers started to market several strains of yeasts dedicated especially to the production of Chardonnay wine.

The classical buttery note of the typical Chardonnay dominates only in wines which have undergone malolactic fermentation after alcoholic fermentation (due to the formation of diacetyl from citric acid), but it is also present in young wines obtained without this second fermentation. Of course, the malolactic fermentation generates a number of other volatile or non-volatile aromatic compounds (van Vuuren and Dicks, 1993), which makes it desirable for this particular white variety, but in this paper we deal only with the differences induced by the alcoholic fermentation with several yeast strains.

The fruity and floral aroma of a wine is mainly due to the terpenic aroma from grapes and esters produced during alcoholic fermentation, therefore the yeast strain and fermentation temperature play a decisive role in the aromatic profile of a wine, sometimes contradicting the influences of the variety and region/terroir.

In this paper, 9 strains of yeast were used to ferment Chardonnay must and the resulted wines were compared by a panel of winetasters and by a gas chromatograph working on the principle of the electronic nose. The wines were evaluated while young, without any oak flavouring from barrel aging or chips usage, thus focusing on the modulation of Chardonnay aroma by yeast selection.

### Materials and methods

#### *Winemaking and Yeasts:*

During 2012 vintage (harvest day was September 14<sup>th</sup>) Chardonnay must from from Murfatlar vineyard, Romania, was fermented with 9 commercially available yeasts, among which some recommended for other grape cultivars than Chardonnay. The wine was obtained in batches of 4 liters each (in triplicate), following a traditional technology for white wines, with skin separation and cold clarification for 48 h before yeast inoculation.

For the alcoholic fermentation the wines were inoculated with 10 g/hl re-hydrated dry yeasts provided by Enologica Vason and Lallemand. The strains were as follows:

Premium Chardonnay: this is a *Saccharomyces cerevisiae* strain, recommended for the production of fine white wines, producing phenylethanol, enhancing floral aroma, but preserving the grape/vineyard aromatic profile;

Premium Blanc 12 V: it is a *Saccharomyces cerevisiae* strain, but with killer phenotype and fast growth, ensuring its rapid prevalence against the wild yeasts. It has  $\alpha$ -glucosidic enzymes, contributing to an increase of the normal aroma of the grape variety, by splitting the glycoside bonds of glucose combined volatile substances, especially of the terpenic ones (technical sheet, [www.vason.com](http://www.vason.com));

Epernay 2: a *Saccharomyces cerevisiae* strain, with low production of unwanted metabolites (acetic acid, acetaldehyde, pyruvic acid, superior alcohols), but able to enhance the various aromatic characteristics of the original must, producing fresher and fruitier wines;

NT116 Anchor: this is an yeast with killer positive phenotype, a *Saccharomyces cerevisiae* hybrid, combining the aromatic potential of a *Saccharomyces cerevisiae* subspecies *cerevisiae* with the fast fermentation capabilities of *Saccharomyces cerevisiae* subspecies *bayanus*. It is not produced by GMO processes, but through a yeast hybridization process, which is a natural method of breeding (van Rensburg P., 2005). Is recommended for the production of fresh and fruity wines, for early release on the market. The producers indicate that “it enhances volatile

thiol aromas (passion fruit, grapefruit and guava) and produces acetate esters (tropical fruit salad). It specifically enhances the citrus aromas in wines”. This yeast is recommended for the winemaking of varieties such as Chardonnay, Chenin blanc, Sauvignon blanc and Pinot gris.

NT202 Anchor: a *Saccharomyces cerevisiae hybrid* strain, recommended for fruity red wines, highly tolerant to ethanol and compatible with malolactic fermentation.

Vin 13: is a an yeast with killer positive phenotype, *Saccharomyces cerevisiae hybrid* strain, from South Africa, designed to enhance the floral and tropical fruits aroma of white wines, suitable for white wines aging and compatible with malolactic fermentation.

Premium Sauvignon (PremSauvig): a *Saccharomyces cerevisiae* strain, producing complex and elegant white wines, especially from aromatic varieties, enhancing the original aroma of the grapes, with an intensification of the floral notes

Lalvin QA23: it is a an yeast with killer positive phenotype classified as a *Saccharomyces cerevisiae bayanus* selected from Portugal, being recommended for the fermentation of Chardonnay, Sauvignon blanc, Chenin blanc, Colombard and Semillon in which it produces fresh, fruity, clean aroma. The yeast has good ethanol tolerance (14%). It is intended to enhance aromas of terpenic cultivars through its  $\alpha$ -glucosydic activity, but it is also an excellent thiol converter, recommended as a complementary yeast for developing passion fruit character in Sauvignon blanc wines.

Bayanus PC: a *Saccharomyces bayanus* strain, dominant in relation with other yeasts, resistant to external adverse factors such as pH, alcohol, SO<sub>2</sub>, conferring a specific volatile profile.

#### *E-nose and Sensory profiles*

An electronic nose based on dual-column flash gas-chromatography, named Heracles, from the Alpha MOS company, was used to differentiate and group the variants based on their volatile profiles (Antoce and Namolosanu, 2011). The analytical method applied is developed in our laboratory (Antoce and Namolosanu, 2009; Antoce *et al.*, 2010; Antoce and Namolosanu, 2011) and uses the following parameters: incubation temperature 60°C, incubation time 600 s, injector temperature 200°C, detector temperature 220°C, measurement time 20 s, trap temperature: initial 40°C and 250°C at desorption, preheating trap time 20 s, baking time 60 s, pre-purging time 5 s. The sampling program of the gas chromatograph starts at a temperature of 40°C maintained for 2 s and raised by 5°C/s up to 200°C where it is also maintained for 5 s, then cooled down. The data acquisition time per sample is 40 s. The volatiles of wines are injected into the GC column by headspace sampling, using a HS 100 autosampler and a syringe of 2500  $\mu$ l, which allows accurate sampling. The data recorded from the two chromatographic columns were processed with the Alpha Soft V11 software for statistical analysis provided by Alpha-MOS with the apparatus. By using the multivariate analysis the accurate differentiation of the samples based on their volatile profile is possible.

Sensory analysis was also performed on all wine variants, using a panel of 15 trained judges recruited from among the master students of University of Agronomical Sciences and Veterinary Medicine of Bucharest majoring in wine technologies, having experience in wine sensory analysis. Each judge evaluated the samples in OIV approved wine glasses containing 20 ml of wine, in random order. The evaluations were performed at 20°C in the time interval from 10:00 to 12:00.

The panelists evaluated the wines focusing mainly on their volatile profile characteristics, using a specially designed score sheet and methodology (Antoce and Namolosanu, 2007). The attributes to choose from were provided to the tasters under the form of a list, and scores were awarded on intensity scales from 1 (minimum) to 9 (maximum) (Muratore *et al.*, 2007).

The set of attributes provided for the evaluation included 21 aroma attributes, as follows: temperate climate fruits (apple, pear, peach, apricot, melon), tropical climate fruits (banana, pineapple, mango/ guava), citrus fruits (lemon, grapefruit, orange), sweet-floral aroma (accacia, hawthorn, honey), nutty aroma (hazelnut, almonds, nuts), lactic aroma (butter, cheese), other aroma (ethyl acetate, mineral). Out of the 21 descriptors, the most common 10, which were marked by most of the tasters, were retained to obtain a consensus-like Chardonnay profile. This is not a true consensus profile, as ours does not come from discussions among members who individually evaluated the wines and then agreed upon a single score for a certain descriptor, but it is an average value of the intensity scores awarded by all the members for the most cited 10 attributes. The data were graphically represented by making use of spider web diagrams.

### Results and discussion

The sensory evaluation of the Chardonnay wines produced with the nine yeasts are presented in Fig. 1 (a-d groups), as spider web diagrams. The aromatic profiles obtained for the wines show that they tend to fall in 3 distinct groups, depending on their perceived aromatic characteristics. We defined the following groups:

the *Saccharomyces cerevisiae cerevisiae* group: Premium Chardonnay, Premium Blanc 12 V and Epernay 2;

the *Saccharomyces cerevisiae hybrids* group: NT116 Anchor, NT202 Anchor and Vin 13;

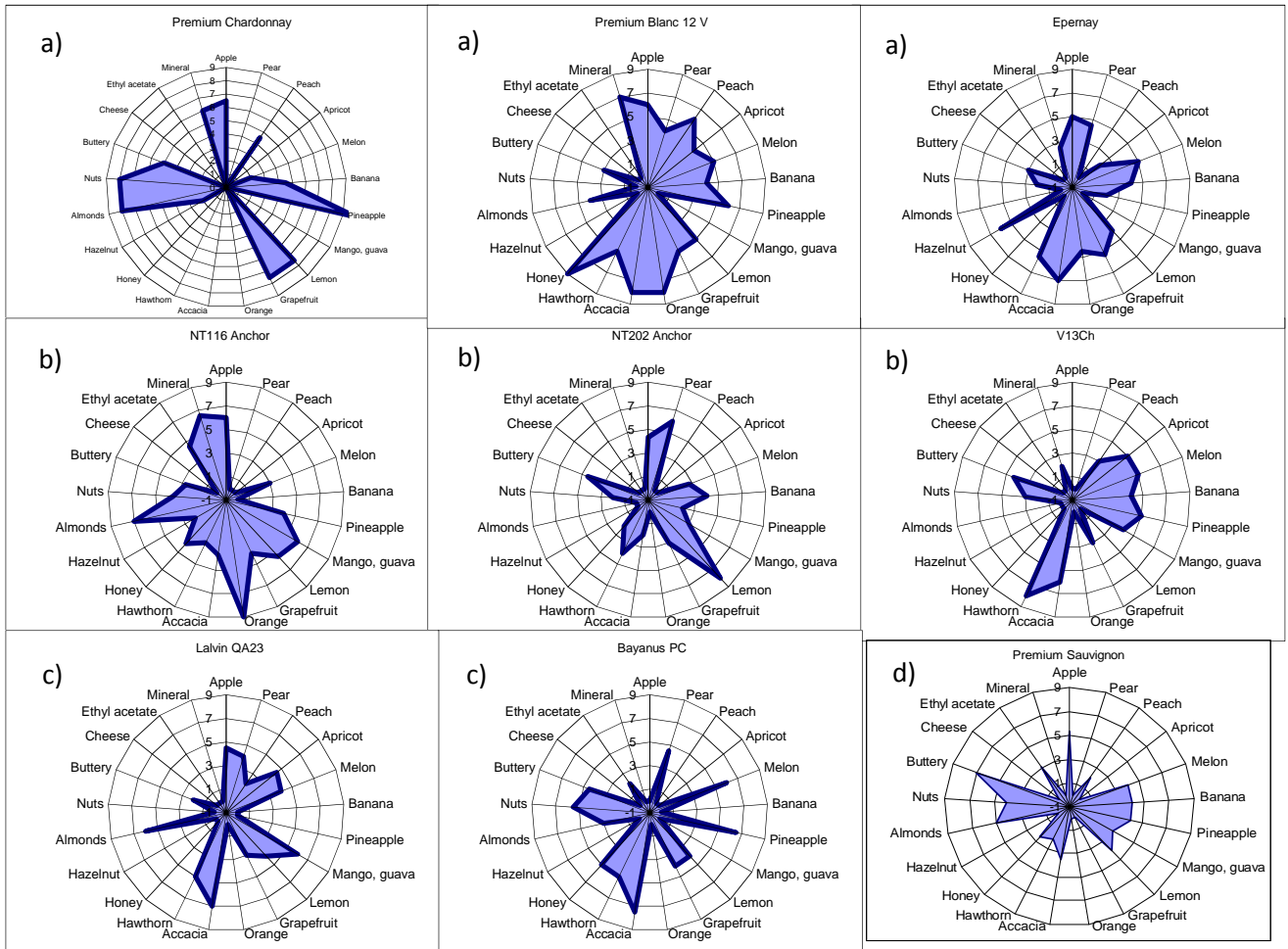
the *Saccharomyces cerevisiae bayanus* group: Lalvin QA23 and Bayanus PC;

plus the *Saccharomyces cerevisiae* strain Premium Sauvignon designed to introduce a certain complexity in wines other than Chardonnay.

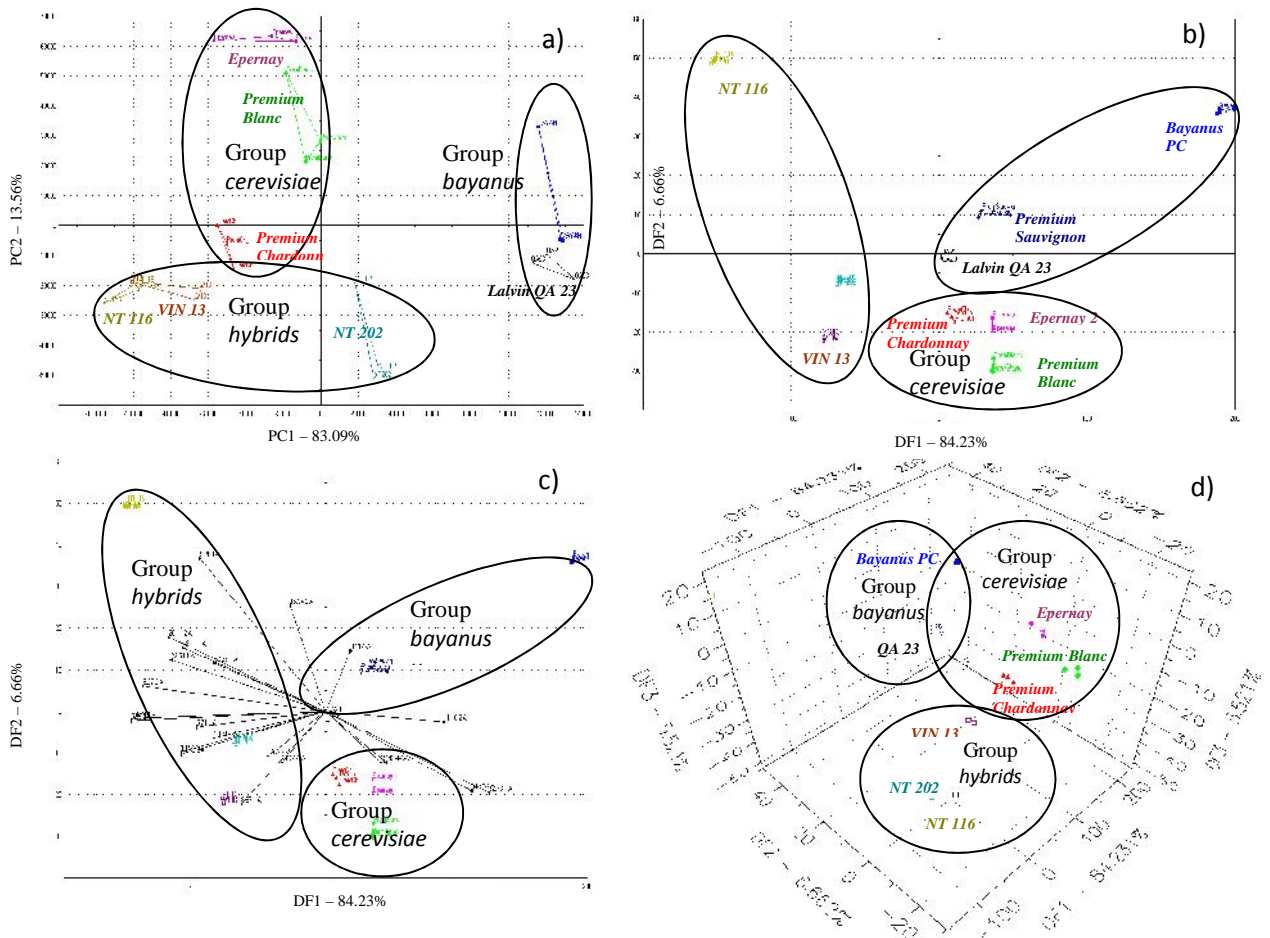
The “*cerevisiae* group” leads to wines with high minerality, aromatic notes of fruits from temperate regions and citrus fruits; the “hybrids group” provides for wines with enhanced tropical fruits aroma and citrus fruits, while the “*bayanus* group” leads to wines with nutty and floral-fruity aroma. The Premium Sauvignon yeast, although it is a *cerevisiae* yeast, being selected for other style of wines and for the production of other volatile compounds or lyses of other type of aroma precursors than those of Chardonnay, produces wines with an aromatic profile closer to those of the “*bayanus* group”.

The same grouping of aromatic profiles is confirmed by the electronic nose both by PCA analysis (Fig 2.a) and DFA analysis (Fig 2.b,c and d). Wines fermented with Premium sauvignon were classified into the group of *bayanus*, due to the complete different aroma profile than that induced by *cerevisiae* yeasts.

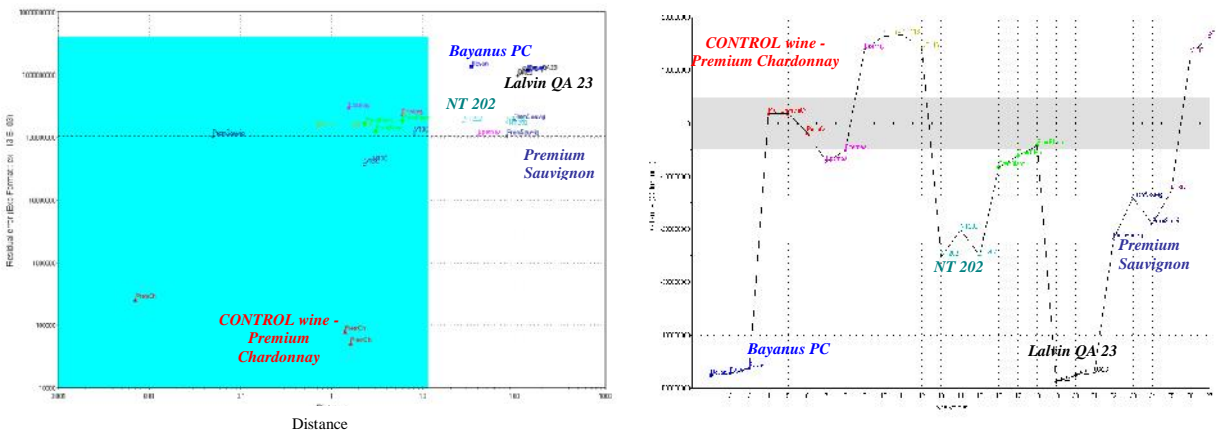
In order to appreciate the odour distances of the wines obtained with other yeasts than the classical Premium Chardonnay, considered here as the control wine, in Fig. 3 we included diagrams for two statistical analyses called SIMCA (Soft Independent Modelling of Class Analogy) and SQC (Statistical Quality Control). Fig. 3 shows that the most different volatile profiles from the classical one obtained by fermentation with Premium Chardonnay yeast are found in the groups obtained with Bayanus PC and QA23 (*bayanus* group) followed by NT202 (hybrid) and Premium Sauvignon strain (*cerevisiae*) both atypical for Chardonnay wines.



**Fig. 1.** The sensory profile of the Chardonnay wines fermented with different selected yeasts: a) *cerevisiae* group (Premium Chardonnay, Premium Blanc 12 V and Epernay 2); b) *hybrids* group (NT116 Anchor, NT202 Anchor and Vin 13); c) *bayanus* group (Lalvin QA23 and Bayanus PC) and d) *Saccharomyces cerevisiae* strain Premium Sauvignon



**Fig. 2.** Classification of Chardonnay wines in accordance to their volatile profile: a) PCA diagram; b) DFA diagram; c) DFA diagram loadings; d) 3-D DFA diagram



**Fig. 3.** SIMCA (a) and SQC (b) diagrams showing the distances of the groups of wines from the control wines produced with Premium Chardonnay yeast strain

Based on both sensory analyses and the confirmation of the classification into groups with similar aroma we were able to establish 3 styles of wines possible to obtain by modulation of the aroma with specific yeast strains. However, in all the wines there are common traits that should also be emphasized. To do this, we proceeded to the reduction of the number of descriptors for the wines and we retained the 10 main attributes that ranked highest during winetasting. The resulted reduced sensory profiles of the Chardonnay wines fermented with different selected yeasts are all included in diagram Fig. 4a, which shows us that the differences among wine profiles were very much reduced, too. By averaging all the scores for these main attributes we can obtain a consensus-like profile of Chardonnay (Fig. 4b) which shows that the main traits of imposed by the terroir on the wine profile are still present in all the produced wines, irrespective of the style induced by the yeast. Thus, we can conclude that the aroma of Chardonnay can be modulated by certain yeast strains, but even so, the characteristics of the Chardonnay cultivar are still recognizable by the tasters.

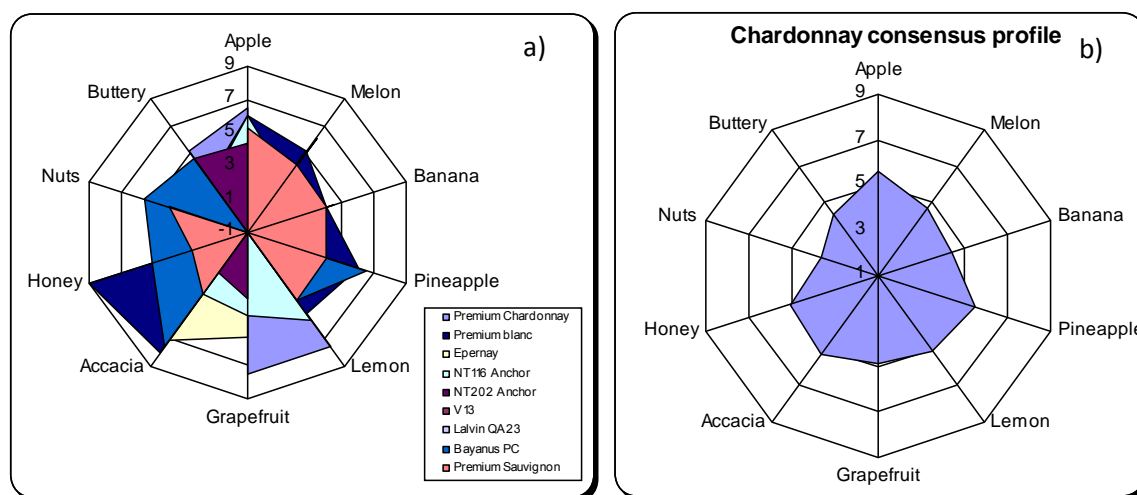


Fig. 4. The reduced profile for the Chardonnay wines (a) and the consensus sensory profile for all the wines, irrespective of the selected yeasts used for fermentation (b)

## Conclusions

The paper evaluates the sensory impact of the yeast strain on the style of Chardonnay wine possible to be obtained in the same terroir. Although the main Chardonnay profile imposed by the origin of grapes was still recognisable in all wines, a modulation of aroma was observed, induced by the fermentation with a certain yeast. We identified 3 main groups of yeasts, which affected the aroma of the wines and lead to obtaining 3 styles of Chardonnay wine. The Chardonnay wines with temperate fruits aroma and citrus were obtained by the fermentation with *Saccharomyces cerevisiae cerevisiae* yeasts; the Chardonnay with tropical fruits aroma and citrus can be produced by fermenting the must with *Saccharomyces cerevisiae hybrid* yeasts, and Chardonnay wines with nutty and floral aroma resulted from the fermentation with *Saccharomyces cerevisiae bayanus* yeasts. These groups of wines were derived by sensory analysis and then confirmed by the results of multivariate analysis performed on the volatile profiles of the wines, obtained with an electronic nose.



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**SEED YIELD OF BIRDSFOOT TREFOIL (*LOTUS CORNICULATUS* L.)  
CULTIVARS IN THE YEAR OF ESTABLISHMENT**

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**Abstract**

Production of sufficient quantities of forage is a prerequisite for the development of livestock production. In South-East Europe in less favorable growing conditions especially in terms of climate and soil, in order to produce sufficient quantities of forage, a special attention is given to the cultivation of the birdsfoot trefoil (*Lotus corniculatus* L.). One of the solutions for improvement of birdsfoot trefoil production is the production of the sufficient quantity of quality seeds. Field trial was established in 2012, on soil type cambisol in a randomized block design with three replications. Cultivars of birdsfoot trefoil (K-37, Rocco and Zora) were sown at a inter row spacing of 20 cm, using 10 kg ha<sup>-1</sup> of seeds. The aim of the study was to analyze in the year of establishment seed yield and yield components: number of stems m<sup>-2</sup>, number of flowers per stem, number of inflorescences m<sup>-2</sup>, number of flowers per inflorescence, number of pods per inflorescence, number of seeds per pod and thousand grains weight. Cultivar Rocco had significantly higher seed yield (408.6 kg ha<sup>-1</sup>) in relation to the cultivars K-37 and Zora (85 kg ha<sup>-1</sup> and 54 kg ha<sup>-1</sup> respectively), which arises from the significantly higher number of flowers per stem and inflorescence m<sup>-2</sup> in relation to the other cultivars. Cultivar Rocco had also a significantly higher number of pods per inflorescence than the K-37 cultivar. Number of flowers per stem, number of inflorescences m<sup>-2</sup> and number of pods per inflorescence were significantly positively correlated with the seed yield.

**Key words:** birdsfoot trefoil, seed yield, yield components.

**Introduction**

Birdsfoot trefoil (*Lotus corniculatus* L.) is a perennial forage legume that is widely distributed in the world. Originates from Western Europe and North Africa (Buselinck and Grant, 1995). In the Republic of Serbia, there are no reliable statistics on the areas where it is grown and yields, although among perennial legumes according to the prevalence it takes the third place, after alfalfa and red clover. It is especially important in the hilly and mountainous areas of Serbia (Petrovic et al., 2011) with regard to its often use when establishing turf in some poor growing conditions (Dimitrova, 2010). According to Vučković (2004) average green forage yields of birdsfoot trefoil range from 35-40 t ha<sup>-1</sup>, and hay from 8-10 t ha<sup>-1</sup>. As a high level in potential of forage yield at this species was obtained, it is also necessary to include monitoring of the most important properties of seed yield and its components. Increasing the potential for seed yield is a rarely important criterion in the early stages of birdsfoot trefoils selection. Breeding for increased potential for seed yield is considerably more difficult due to the lack of clear interdependence between seed yield and its components. On the other hand, the existence of significant genetic correlation between harvest index and seed yield suggests the possibility of increasing seed yield, without affecting the forage yield (Elgersma and Van

Wijk, 1997). Seed yield of perennial legumes is mainly determined by genetic base of the variety, environmental conditions of the area, the moment of removing the first cut, the presence of the pollinating insects and the interaction between genotype and environment (Steiner et al. 1995). Potential seed yield of birdsfoot trefoil is estimated at 1200 kg ha<sup>-1</sup>, while the average yields on global level are below 200 kg ha<sup>-1</sup> (Turkington et Franco, 1980; Gullien, 2007). In the Republic of Serbia, the average seed yields range from 100-280 kg ha<sup>-1</sup> (Vu koviC et al., 1997). According to Miladinovic (1967) with the full agricultural technology in our conditions the seed yields of birdsfoot trefoil can reach more than 350 kg ha<sup>-1</sup>.

The aim of the research was to make a selection of the birdsfoot trefoil cultivars with higher potential for seed yield, out of the chosen cultivars, by analyzing seed yield and its components. Such genotypes would represent a good basis for further work on hybridization and the creation of new varieties, which would have higher seed yield, beside the high potential for forage yield.

### Material and Method

The experiment was established near the town of Ljig, in 2012 on the soil in type of cambisol (according to the WRB classification), with medium amounts of nutrients. Primary tillage was conducted at a depth of 30 cm. Along with the primary tillage, the soil was entered 300 kg ha<sup>-1</sup> N<sub>15</sub>P<sub>15</sub>K<sub>15</sub>. Field experiment with three varieties of birdsfoot trefoil was set up using a randomized block design with three replications with plot size 5 m<sup>2</sup> (5x1m). The cultivars of birdsfoot trefoil: K-37 (Institute for forage crops Krusevac), Zora (Institute for agriculture and technological researches Zaje ar) and Rocco (Italian cultivar) were sown at the inter row spacing of 20 cm, with the seed amount of 10 kg ha<sup>-1</sup>. Weed control was done mechanically on two occasions. Plants were grown without irrigation.

Seed yield and seed yield components were determined from the first cut in the year of the establishment. The following components of yield were determined on the field: number of stems per m<sup>-2</sup>, number of inflorescences per m<sup>-2</sup> (by counting on the area of 0.2 m<sup>2</sup> per elementary plot), inflorescences number per stem and number of pods per stem (counting on ten randomly selected tillers from the elementary plot). In laboratory were determined: number of flowers per inflorescence, number of pods per inflorescence and number of seeds per pod (a sample of ten inflorescences per elementary plot) and thousand grains weight (based on the weight 5x100 of seed). The actual seed yield was determined based on the yield components (number of inflorescences per unit area, number of pods per inflorescence, number of seeds per pod, weight of thousand grains) and converted to seed yield in kg ha<sup>-1</sup>.

The obtained results were processed by analysis of variance of the single factorial experiment using the SPSS software (1995). Significance of differences between the mean values was tested using LSD test.

### Results and discussion

The varieties didn't differ among themselves in the number of stems per m<sup>-2</sup>, while there were significant differences between varieties recorded in the number of inflorescences per stem, which resulted differences in the number of inflorescences per m<sup>2</sup>. A significantly higher number of inflorescences per stem and inflorescences per m<sup>2</sup> in relation to the other cultivars had the cultivar Rocco. McGraw et al. (1986a) indicate that the number of flowers per stem is the component of yield that the yield of birdsfoot trefoil is largely dependent on. Varieties had an average of 3.21 of flowers per inflorescence and 1.62 pods per inflorescence. Cultivar Rocco had a significantly higher number of pods per inflorescence than K-37.

The number of seeds per pod ranged from 8 in cultivar Zora to 10.22 in cultivar Rocco and thousand grain weight from 1.22 g in cultivar Zora to 1.25 g in cultivar K-37. Grant (1967) states that one gram has 750 to 800 grains of birdsfoot trefoil seed, or the mass of thousand grains is about 1.3 g. According to Hansen (1953) the average number of seeds per pod of birdsfoot trefoil genotypes was 19.

In full maturity pods easily shatter and seeds effuse. Shattering problem significantly limits the successful production of seeds (Fairey, 1994). According to Winch et al. (1985) losses in harvesting birdsfoot trefoil seeds are large, ranging up to 85 % of potential yield. In this paper, the birdsfoot trefoil seed yield is presented, recalculated on the basis of yield components, without taking into account the losses at harvest. The highest yield had variety Rocco (408.6 kg ha<sup>-1</sup>), significantly higher than the cultivar K-37 (85.0 kg ha<sup>-1</sup>) and Zora (54.0 kg ha<sup>-1</sup>). This is mainly due to a larger number of inflorescences per stem and number of pods per inflorescence in cultivar Rocco in relation to the others. Potential seed yield of birdsfoot trefoil is estimated at 1200 kg ha<sup>-1</sup>, while the average yields on the global level are below 200 kg ha<sup>-1</sup> (Turkington et Franco, 1980; Gullien, 2007). According to McGraw and Beuselinck (1986a), the average yield of birdsfoot trefoil seed varies from 50-175 kg ha<sup>-1</sup>, or in average it is about 100 kg ha<sup>-1</sup> of seed. Seaney and Henson (1970) state that seed yields of birdsfoot trefoil range from 50-560 kg ha<sup>-1</sup>. Seed yields of birdsfoot trefoil in USA range from 50 to 170 kg ha<sup>-1</sup> (Fairey and Smith, 1999), in Uruguay between 120 and 150 kg ha<sup>-1</sup> (Garcia et al., 1991; Artola, 2004) and in Argentina between 25 and 150 kg ha<sup>-1</sup> (Mazzanti et al., 1988). According to Vojin et al. (2001), in agro-ecological conditions of Serbian republic in the area of Banja Luka, there was a birdsfoot trefoil seed yield of 272 kg ha<sup>-1</sup>. In Serbia, seed yield ranges from 100-280 kg ha<sup>-1</sup> (Vu koviC et al., 1997).

Birdsfoot trefoil can achieve good yields in seed production (PetroviC, 2011), so in combination with two cuts, considerable yield can be achieved for forage. Autor observed that highest yields of forage in the densest crop establishment do not match the highest seed yield in three-year production, but they have the highest potential in combined use.

Analyzing the seed yield of birdsfoot trefoil at three locations McGrew et al. (1986b) found that there were significant interactions genotype x environment, so the testing of the potential for seed yield should be done in an environment where the seed is commercially produced.

Table 1. Mean seed yield and yield components of birdsfoot trefoil varieties: stem number m<sup>-2</sup> - SNM, inflorescence number per stem – INS, inflorescence number m<sup>-2</sup> – INM, flower number per inflorescence – FPI, pod number per inflorescence – PNI, seed number per pod – SNP, thousand seed weight - TSW and seed yield - SY (g).

		SNM	INS	INM	FPI	PNI	SNP	TSW	SY
Cultivars	K-37	450	0.89 b	415 b	3.5	1.80 b	9.57	1.24	85.0 b
	Rocco	428	3.50 a	1464 a	2.7	2.20 a	10.22	1.23	408.6 a
	Zora	513	0.58 b	297 b	3.4	1.87 ab	8.00	1.22	54.0 b
ANOVA		ns	*	*	ns	*	ns	ns	*

The values denoted with different small letters within columns are significantly different at (P<0.05) in accordance with the LSD test; \*- F test significant at p<0.05; ns - F test non-significant.

Table 2. Correlation coefficients between stem number  $m^{-2}$  - SNM, inflorescence number per stem - INS, inflorescence number  $m^{-2}$  - INM, flower number per inflorescence - FPI, pod number per stem - PNS, pod number per inflorescence - PNI, seed number per pod - SNP, thousand seed weight - TSW and seed yield - SY.

	INS	INM	FPI	PNS	PNI	SNP	TSW	SY
SNM	-0.42	-0.09	-0.12	-0.54	0.0	-0.38	-0.05	-0.04
INS		0.93*	-0.47	0.44	0.66	0.54	-0.02	0.89*
INM			-0.46	0.39	0.76*	0.51	0.01	0.99*
FPI				0.09	-0.08	0.11	0.02	-0.38
PNS					0.2	0.76*	0.05	0.38
PNI						0.26	-0.27	0.77*
SNP							0.09	0.54
TSW								0.05

\* Significant at  $p < 0.05$

Number of inflorescences per stem was strongly positively correlated with the number of inflorescences per  $m^{-2}$ , where both features positively correlated with the seed yield. A positive correlation was also found between the number of pods per inflorescence and the number of seeds per pod and number of pods per inflorescence and seed yield. According to StevoviC et al., (2011) the number of inflorescences per plant of red clover is positively correlated with the number of stems per plant and number of inflorescences per stem. The authors also reported a significant positive correlation between the number of inflorescences per plant and seed yield per plant. These results are in agreement with results of Montardo et al., (2003) and Herrmann et al. (2006). Nevertheless, Herrmann et al., (2006) report that the number of inflorescences per plant largely determines the seed yield of red clover.

### Conclusion

Significant differences in the number of inflorescences per stem, inflorescence number per  $m^{-2}$ , number of pods per inflorescence and seed yield were detected among the cultivars. Cultivar Rocco had significantly higher seed yield ( $408 \text{ kg ha}^{-1}$ ) in relation to K-37 and Zora ( $85 \text{ kg ha}^{-1}$  and  $54 \text{ kg ha}^{-1}$ , respectively) as a result of a significantly higher number of inflorescences per stem and number of inflorescences per  $m^{-2}$  in relation to the others. Cultivar Rocco also had a significantly higher number of pods per inflorescence than K-37. The values of correlation coefficients indicate that the greatest influence on the yield had the following yield components: number of inflorescences per stem, number of inflorescences per  $m^{-2}$  and number of pods per inflorescence, so a further selection work should focus on increasing the value of these properties.

The results indicate that seed production of birdsfoot trefoil can be successful in the year of sowing with proper care and use of crop.

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**MALFORMATIONS OF REPRODUCTIVE ORGANS IN WALNUT  
(*JUGLANS REGIA* L.)**

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**Abstract**

In this research, which was conducted in *native walnut population*, during 2000-2011 years in the region of Kraljevo (central Serbia), more than 2000 *walnut* seedling trees were observed. In some trees unusual phenomena in the structure and function of reproductive organs were expressed, such as: second and successive flowering, mixed inflorescences with female flowers at its base and male flowers at the top, hermaphrodite flowers, “V” shaped catkins, spurs only with catkins, flowers and fruits fusion, irregular shell and kernel segmentation and *incomplete involucre*s. Certain of these malformations occur in some trees every year, while in some cases they represent a sporadic phenomenon, probably caused by environmental factors. In this paper all these phenomena are described in detail and documented by photographs.

**Keywords:** *walnut, reproductive organs, malformations.*

**Introduction**

Walnut growing has a long tradition in Serbia, where the number of bearing trees is around two million. The soil and climatic conditions in most regions are suitable for walnut cultivation, and walnut trees are found in all fruit-growing regions. It has been propagated from seed for centuries, resulting in a heterogeneous population in which each tree is a different genotype (Cerovic et al., 2010).

Walnut (*Juglans regia* L.) is monoecious plant, and its male and female flowers are born separately on the tree. It normally develop unisexual inflorescences (Hickey and King, 1981). The male flowers are densely packed on catkins, which hang from the tree in the spring. Each catkin has up to 40 sessile petalless florets, each with numerous stamens. The immature naked catkin buds first appear in leaf axils in late summer and persist over winter, maturing in the spring in the axils of leaf scars on wood from the previous season. Female flowers are borne on the current season's growth in spikes of two to five flowers. Flowers are typically produced on the tips of terminal shoots shortly after leaves emerge. The female flower's entire basal portion is enclosed within a hairy involucre formed by the fusion of several accessory flower parts (McGranahan and Leslie, 2009). Inside the involucre is the pistil, which has a swollen base (the ovary), and a short style, with a forked stigma with two feathery stigmatic lobes. The ovary is surrounded by the ovary wall. Attached to the ovary is a single ovule enclosed by a single integument (Polito, 1998; Pinney et al., 1998). The fruit is a nut enclosed in an indehiscent, thick husk. The fertilized egg is zygotic tissue and becomes the embryo and later the edible kernel. The kernel consists of two fleshy cotyledons and an embryonic axis (Janick and Paull, 2008).

Abnormal flowers have been recognized as curiosities by botanists for more than two thousand years (Meyerowitz et al., 1989). It can be induced by several environmental



adversities and by genetic factors. The knowledge of genetic relationships among walnut genotypes and their reproductive characteristics are really useful in walnut cross-breeding programs (Cosmulescu and Botu, 2012). The objective of this paper is to present various anomalies observed on the reproductive organs of walnut *during the eleven-years period of investigation* of the native walnut population in the Kraljevo area.

### Materials and methods

The study was conducted in *native walnut population in several localities in the region of Kraljevo* (central Serbia), during the period 2000 - 2011. More than 2000 *walnut* seedling trees were observed. The main objective of this study was to examine the phenological and reproductive traits of walnut trees in spontaneous habitats and selection of interesting genotypes. The study included *sampling and observation of collected plant material*. During this study various anomalies of the walnut reproductive organs *have been* identified, which has *been* photographed and described.

### Results and discussion

#### *Apomixis*

Apomixis in flowering plants is defined as the asexual formation of a seed from the maternal tissues of the ovule, avoiding the processes of meiosis and fertilization, leading to embryo development. Therefore, this embryo has a genetic constitution identical to that of the female parent (Bicknell and Koltunow, 2004). Apomixis is present in walnut. *Rate of apomictic fruits* in walnut differs from year to year and depends on climate and genotype (Asadian and Pieber, 2005). The mechanism of apomixis in walnut has been reported as adventitious embryony (Valdivieso, 1990), apospory (Terziiski and Stefanova, 1990), or diplospory (Sartorius and Stosser, 1991). We identified apomixis in several walnut genotypes during the period of examination. In all these cases we have recognized the apospory (Fig. 1-a).

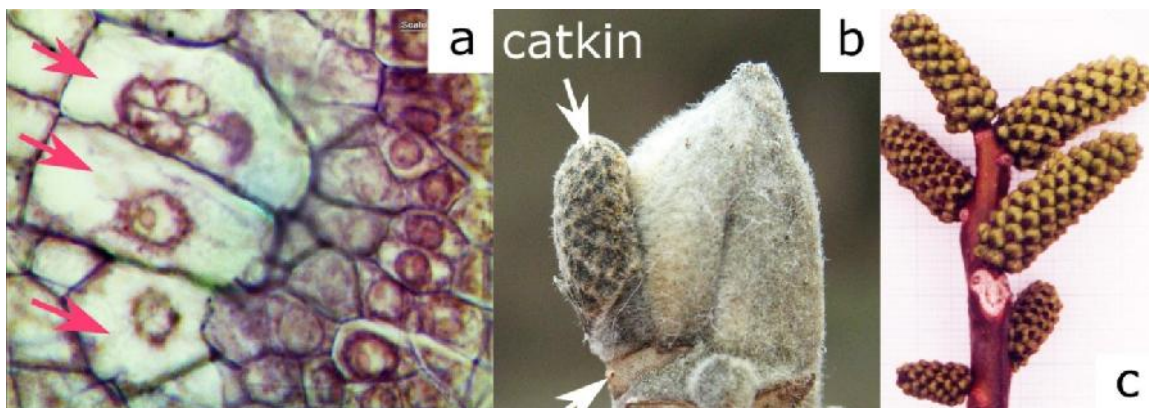


Figure 1: a) *aposporic embryo sacs* in the walnut nucellus; b) catkin formed in the axill of the terminal bud scale; c) “male spur”.

#### *Catkin development from the terminal growing point of the shoots*

Male flowers of walnut are grouped in catkins, which develop laterally on the previous season’s growth (Polito, 1985). On the adult trees, monocyclic shoots (that are formed by the first growth flush in spring) are usually female, flowering in the terminal position (Solar and Štampar, 2003). In some genotypes catkins are formed in the terminal position of the shoots.

These genotypes are prone to form “male spurs”, which carry almost no other buds than catkins. Sometimes, a catkin is also formed in the axill of the terminal bud scales (Fig. 1-b, c).

*Occurrence of the “V” shaped catkins*

In the year 2002 almost all catkins from one solitary walnut tree were “V” shaped. We did not detect reason of that phenomenon, but it is certain that it was induced by environmental factors (Fig. 2-a).

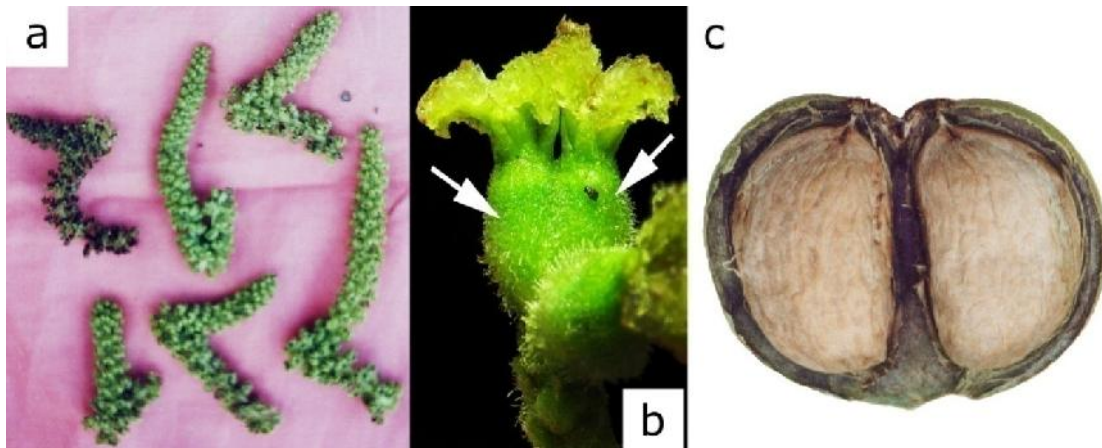


Figure 2: a) “V” shaped catkins; b) female flower with double pistils; c) twin fruits developed from a female flower with double pistils.

*Doubling of pistils*

Surányi (1979) studied floral anomalies of several *Prunus* species and found on average, 1,7% twin pistils. High summer temperatures at the time of flower bud differentiation are believed to cause forming of double pistils, resulting in many double or malformed fruits at harvest (Micke et al., 1983). Besides the normal flowers, with two stigmas, Kholdorov (1975) noticed the walnut flowers with three stigmas in some genotypes. In our examination, we found several examples of double pistils (Fig. 2 - b, c), but this abnormality was not common.



Figure 3 – second flowering in walnut: a) hermaphrodite inflorescence; b) female inflorescence; c) hermaphrodite flowers; d) hermaphrodite inflorescence with mature fruits.

*Second flowering and hermaphroditism*

In some walnut trees, after the normal flowering, secondary flowering waves are also identified during a growing season. Walnut normally develops unisexual inflorescences, but *in case of secondary flowering* inflorescences may carry male, female and hermaphrodite flowers. Female inflorescences are grouped at the top of relatively small shoots with very short internodes (Fig. 3 - a). *On the hermaphrodite inflorescences* female flowers are placed at its base and male flowers at the top (Fig. 3 - a, d). Sometimes on the mixed inflorescences partially developed hermaphrodite flowers were placed, with a pistil in the centre and the stamens around it (Fig 1 - c). Similar phenomena have been described by Germain et al. (1997) and Breton et al. (2004) in some walnut seedlings originating from central Asia.

*Abnormalities in fruit development*

As the fruit matures, the involucrem and fused sepals develop into the husk (hull), and the shell is formed from the ovary wall. Walnut fruit contain two kernels separated by a thin, papery central plate, extending from the inner layer of the shell. Two primary (major) septa grow inwards from the point of union of the two carpels and unite the lower part of the fruit to a varying degree. The major septum is in the plane perpendicular to the suture lines. Two secondary (minor) septa may also develop in the lower part of the endocarp, causing the seed to become four-lobed at its base. The minor septum lies in the plane of the shell sutures. The endocarp dehisces along the two prominent midribs situated opposite the two cotyledons. *In this study we noted three genotypes which form abnormal fruits. Two of them had the fruits with irregularly formed sutures on the shell and septa (nuts with ½, 1, 2, 3, 4, and 5 sutures) (Fig. 4-b). The third genotype formed fruits with incomplete involucrem, below which the sepals are visible (Fig. 4-a).*

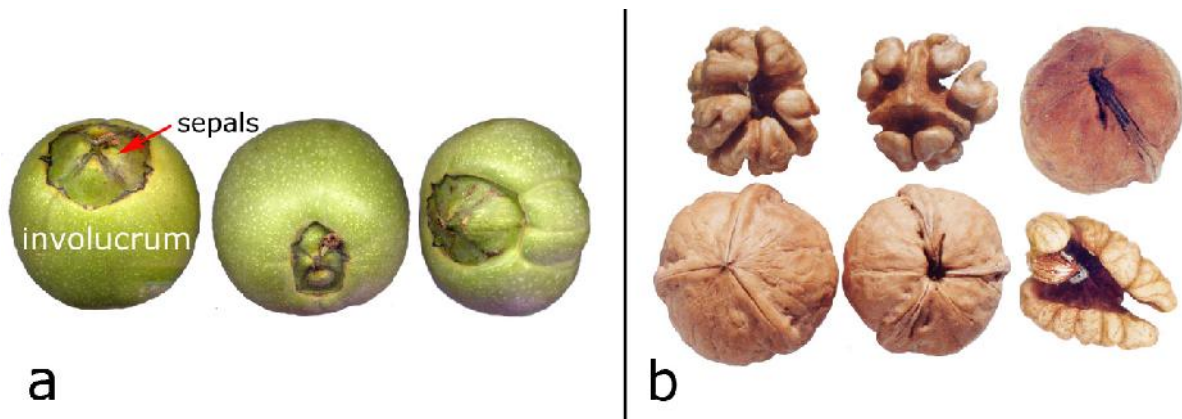


Figure 4: a) fruits with *incomplete involucrem*; b) fruits with *irregularly formed sutures on the shell and kernels*.

**Conclusion**

During the period 2000-2011 in the *native walnut population* in the region of Kraljevo unusual phenomena in the structure and function of reproductive organs were expressed such as: second and successive flowering, mixed inflorescences with female flowers at its base and

male flowers at the top, hermaphrodite flowers, “V” shaped catkins, spurs only with catkins, flowers and fruits fusion, irregular shell and kernel segmentation and *incomplete involucre*s. Certain of these malformations occur in some trees every year, while in some cases they represent a sporadic phenomenon, probably caused by environmental factors. In the case of the heritable abnormalities it is possible to study gene interactions and gene dosage effects when they influence the degree of abnormality expressed by the plant.

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## PHENOLOGY AND YIELD OF NINE SOUR CHERRY CULTIVARS UNDER CENTRAL SERBIA CONDITIONS

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### Abstract

Phenological properties (beginning of flowering, full flowering, end of flowering, beginning of fruit colouring and harvest date) and yield of nine sour cherry cultivars, grafted on *Prunus avium* seedling rootstock, were studied in the region of Kraljevo (central Serbia), during a ten – year period (2000-2010). The mean date of beginning of flowering was 12 April, full flowering 17 April, and the end of flowering April 23. Flowering lasted, on the average, 11 days. Difference in flowering dates of the earliest and the latest flowering cultivars was 10 days, and the time span between years was 18 days. Early flowering was observed in cultivars ‘Richmorency’, ‘Obla inska’, ‘Heimanns Rubinweichsel’ and ‘Lešanski Rubin’, followed by ‘Heimanns Konservenweichsel’, ‘Kelleriis 16’ and ‘Rexelle’, while late flowering was observed in ‘Schattenmorelle’ and ‘Kelleriis 14’. The time span between the cultivars of the earliest and the latest average fruit ripening was approximately 16 days, and the biggest difference between years with the earliest and the latest average fruit ripening was 22 days. ‘Richmorency’ and ‘Obla inska’ are characterized by middle-early ripening (25-30 June), followed by medium late cultivars, such as ‘Lešanski Rubin’, ‘Heimanns Rubinweichsel’, ‘Rexelle’, ‘Kelleriis 16’ and ‘Heimanns Konservenweichsel’ (1-2 July). ‘Schattenmorelle’ and ‘Kelleriis 14’ proved to be late maturing (8-9 July). The average annual yield per tree amounted to 14-28 kg. The highest cumulative yield per tree was recorded in ‘Rexelle’ (224 kg), and the lowest in ‘Kelleriis 16’ and ‘Schattenmorelle’ (113.2 kg).

**Keywords:** sour cherry, cultivars, flowering, fruit maturation, yield.

### Introduction

The sour cherry belongs to a group of high quality delicacy fruit. It has significant nutritional, medicinal, dietary and technological value. It is used as fresh fruit, or as raw material in processing industry, mostly in juices, jams, jelly, yoghurt, marmalade, brandy, liqueurs, compotes, as well as raw material in confectionery industry (Cerovic et al., 2005). The Republic of Serbia has very favorable natural and climatic conditions for sour cherry growing. The sour cherries are important and highly perspective Serbian fruits, primarily from the aspect of export on the international market. In the structure of fruit growing in Serbia, sour cherry ranks third, with 8.7 million trees and production of 89,746 t in 2008, and Serbia is ranked seventh in the world in the production of sour cherries, with a share of around 7% (Sredojevic, 2011). Sour cherry is one of the most important export products of Serbia, the greatest part being produced as frozen and subsequently exported (Radi evic et al., 2012). The current assortment of sour cherries is directed mainly to the cultivars that are used for industrial processing. The most represented cultivars in Serbia are ‘Obla inska’ (accounting for 60% of the total number of cherry trees) and the spontaneously spread ‘Cigan ica’ (20%).

Other cultivars represented are ‘Heimanns Konservenweichsel’, ‘Rexelle’, ‘Šumadinka’ and ‘Schattenmorelle’, all accounting for 20%. ‘Schattenmorelle’ and ‘Heimanns Konservenweichsel’ are also grown in many countries as a highly valued cultivars (Nikolic and Milatovic, 2011).

Knowledge of ecophysiological and economic characteristics of genotypes of fruit trees is a basis for assessing the suitability of their growing in certain environmental conditions and defining the appropriate cultivar specific cultural practices. The objective of this study is to determine the phenological characteristics and yield of nine sour cherry cultivars in the ecological conditions of Kraljevo (central Serbia).

### Materials and methods

The present study was carried out at village Dedeveci (43°43' N, 20°29' E, about 392 m above sea level), near Kraljevo (central Serbia), during a ten-year period (2000-2010) on nine sour cherry cultivars: ‘aanski Rubin’, ‘Heimanns Konservenweichsel’, ‘Heimanns Rubinweichsel’, ‘Kelleriis 14’, ‘Kelleriis 16’, ‘Obla inska’, ‘Richmorency’, ‘Rexelle’ and ‘Schattenmorelle’. All cultivars were grafted on *Prunus avium* seedling rootstock. The orchard was built in the fall of 1997. The trees were planted at a spacing 5 x 3 m. Five representative trees within each replicate were selected for sampling and data collection. The five replicates were arranged in a randomized completely block design. Trees were trained to the standard open vase system. Standard cultural practices, such as fertilization, pruning, pest control and soil management were performed annually, in accordance to normal commercial practices, except irrigation. Fertilizers were applied according to soil analyses. The orchard was clean cultivated during the complete investigation period.

Weather conditions of Kraljevo are characterized by the average annual temperature of 11°C and total annual rainfall of 720 mm. The soil texture in the orchard is clay-loam. The content of organic matter in 0–30 cm soil depth was 2.8%, 13.4% P<sub>2</sub>O<sub>5</sub> and 21.1% K<sub>2</sub>O. Soil pH in 0,01 M KCl was 5.8.

The phenological characteristics of cherry trees have been studied as follows:

beginning of flowering – when 10% of flowers were open (flowers are considered to be opened if the anthers and pistils are easily recognized from above and the stigma is green or yellow);

full flowering – when 80% of flowers were open;

end of flowering – when 90% of petals were fallen;

beginning of fruit colouring (when the fruit skin begins to change color from green to red);

fruit ripening time (was determined as the day when the fruits were sufficiently coloured and soft for eating).

The yield (per tree and per hectare) was studied from the sixth year after planting (when the trees reached full production) up to the thirteenth year. The data obtained from yield measurements are processed by the statistical method of the analyses of variance. The significance of differences between mean values is determined by Tukey’s test at P=0.05.

## Results and discussion

### Bloom time

The results of the study of the bloom time of sour cherries are shown in the table 1. The average date for the beginning of blooming of all tested cultivars was April 12, for the full bloom April 17, and for the end of blooming April 23. The interval from the beginning to the end of blooming is 11 days and from the full bloom to the end of blooming six days.

The earliest sour cherry blooming time was recorded in the year 2007, when the beginning of blooming was on April 07, full bloom on April 11, and the end of blooming on April 18. The latest blooming time was in the year 2005, when the beginning of blooming was on April 25, full bloom on April 29, and the end of the blooming on May 06. The difference in bloom time between the years with the earliest and the latest recorded blooming is 18 days.

The cultivars 'Richmorency' and 'a anski Rubin' had the earliest average beginning of blooming (April 10), while the cultivar 'Kelleris 14' had the latest (April 20), so the difference between cultivars with the earliest and the latest beginning of blooming was 10 days. Based on these data, the examined cultivars can be classified into the following groups: 1) early blooming cultivars: 'Richmorency', 'Obla inska', 'Heimmanns Rubinweichsel' and 'a anski Rubin'; 2) middle blooming cultivars: 'Heimmanns Konservenweichsel', 'Rexelle' and 'Kelleris 16'; 3) late blooming cultivars: 'Schattenmorelle' and 'Kelleris 14'. Similar conclusions were reached by Stan evic (1969) in the area of a ak, but Mišic (2002) classified cultivars 'Rexelle', 'Obla inska', 'a anski Rubin' and 'Heimmanns Konservenweichsel' in the middle blooming group, and 'Schattenmorelle', 'Kelleris 14' and 'Kelleris 16' in the group of late blooming cultivars. Blasse (1964) and Kellerhals (1986) also classified 'Schattenmorelle' in the late blooming group. According to several authors (Milutinovic et al., 1980; Nikolic et al., 2005; Rade et al., 2008; Rakonjac et al., 2010), 'Obla inska' represents a heterogeneous population, which is a mixture of numerous genotypes. Fotiric Akšic et al. (2013) observed genetic diversity among the 41 genotypes of 'Obla inska' for several traits, including flowering time, maturing time and yield. According to the authors, most of the 'Obla inska' genotypes flowered during April 16 and 17, which is consistent with our results.

Table 1. Flowering of the studied sour cherry cultivars

Cultivar	Begining of flowering		Ful flowering (average)	End of flowering		Duration of flowering (days)
	Earliest date	Average date		Average date	Latest date	
Richmorency	04. IV	10. IV	14. IV	20. IV	03. V	11
a anski Rubin	05. IV	10. IV	14. IV	20. IV	03. V	11
Obla inska	05. IV	11. IV	15. IV	21. IV	04. V	11
Heimmanns Rubinweichsel	04. IV	11. IV	16. IV	22. IV	03. V	12
Heimmanns Konservenweichsel	06. IV	12. IV	16. IV	22. IV	06. V	11
Rexelle	07. IV	13. IV	18. IV	24. IV	07. V	12
Kelleris 16	08. IV	15. IV	18. IV	24. IV	08. V	10
Schattenmorelle	10. IV	18. IV	23. IV	29. IV	13. V	12
Kelleris 14	13. IV	20. IV	24. IV	30. IV	14. V	11
The average for all cultivars	07. IV	12. IV	17. IV	23. IV	07. V	11



## 2. Fruit ripening time

The average date of the beginning of ripening for all examined cultivars was June 19. The earliest average date of the beginning of ripening was in the cultivar ‘Richmorency’ (June 15), and the latest in the cultivar ‘Schattenmorelle’ (June 26), so the time range between the cultivars with the earliest and the latest beginning of ripening was 11 days (Table 2). The earliest average beginning of ripening was on June 15 (2007), when the time range between the earliest and the latest cultivars was 22 days, and the latest average beginning of ripening was on June 27 (2005), with a time range of 14 days between cultivars.

Table 2. Fruit ripening time of the studied sour cherry cultivars

Cultivar	Beginning of fruit colouring		Ripening time		Interval from full bloom to ripening
	Earliest date	Average date	Earliest date	Average date	
Richmorency	10. VI	15. VI	19. VI	25. VI	72
Obla inska	11. VI	16. VI	22. VI	28. VI	74
a anski Rubin	13. VI	18. VI	26. VI	30. VI	77
Rexelle	14. VI	18. VI	23. VI	01. VII	74
Heimanns Rubinweichsel	14. VI	19. VI	24. VI	01. VII	76
Kelleriis 16	14. VI	18. VI	26. VI	01. VII	74
Heimanns Konservenweichsel	15. VI	19. VI	25. VI	02. VII	77
Kelleriis 14	20. VI	24. VI	01. VII	08. VII	77
Schattenmorelle	22. VI	26. VI	03. VII	09. VII	77
The average for all cultivars	15. VI	19. VI	25. VI	01. VII	75,3

Full ripening occurred approximately 12 days after the change in fruit skin colour. The period between the cultivar with the earliest (‘Richmorency’) and the latest full fruit ripening (‘Schattenmorelle’) was 14 days.

The earliest fruit ripening was recorded in 2007, and the latest in 2005, when the time range in fruit ripening was 12-16 days. During the entire period of the examination there were no variations in the order of the fruit ripening in cultivars. Based on the fruit ripening period, the studied cultivars can be classified into following groups: 1) medium early cultivars (‘Richmorency’ and ‘Obla inska’); 2) medium late cultivars (‘a anski Rubin’, ‘Heimanns Rubinweichsel’, ‘Rexelle’, ‘Kelleriis 16’ and ‘Heimanns Konservenweichsel’); and 3) late cultivars (‘Kelleriis 14’ and ‘Schattenmorelle’). Milatovic et al. (2011) classified ‘Obla inska’, ‘a anski Rubin’, ‘Richmorency’ and ‘Rexelle’ in the group of middle ripening cultivars, that ripen in the second half of June, and ‘Schattenmorelle’, ‘Kelleriis 14’ and ‘Heimanns Konservenweichsel’ as the late ripening cultivars, with ripening time from late June till early July. According to Radi evic et al. (2010), the average ripening date for ‘Heimanns Konservenweichsel’ in ecological conditions of a ak for the research period 2006-2007 was 28 June, that is consistent with our results. Fruits of ten examined ‘Obla inska’ sour cherry clones averagely ripened at „Radmilovac“ (near Belgrade) in the three years of the experiment (2000-2002) in first decade of June (Nikolic et al., 2005). That is much earlier than in our experiment, which is a consequence of different ecological conditions in Belgrade and Kraljevo.

## 3. Yield

The average yield of the studied sour cherry cultivars varied between 14.2 kg and 33.6 kg per tree, or 9424 kg and 18648 kg per hectare. The largest yield was recorded in the cultivars ‘Rexelle’ and ‘Heimanns Konservenweichsel’, and the smallest in ‘Schattenmorelle’, ‘Kelleriis 16’ and ‘Richmorency’. Based on the yields obtained per tree, the cultivars can be classified into following groups: 1) high yielding cultivars (over 21 kg per tree) – ‘Rexelle’, ‘Heimanns Konservenweichsel’ and ‘Heimanns Rubinweichsel’; 2) medium yielding cultivars (18-21 kg per tree) – ‘Obla inska’, ‘Kelleriis 14’ and ‘ a anski Rubin’; 3) low yielding cultivars (14-16 kg per tree) – ‘Richmorency’, ‘Kelleriis 16’ and ‘Schattenmorelle’.

Table 3. Yield of the studied sour cherry cultivars

Cultivar	Average yield per tree (kg)								Cumulative yield per tree for eight years (kg)	Average yield for eight years (kg)	
	2003	2004	2005	2006	2007	2008	2009	2010		Per tree	Per hectare
Richmorency	15.4	24.2	31.4	30.5	29.0	35.4	24.5	33.6	224.0	28.0 a	18 648
Obla inska	15.5	22.2	27.6	27.2	25.8	31.6	24.4	30.2	204.5	26.0 ab	17 024
a anski Rubin	12.3	20.5	23.7	25.6	20.2	19.8	20.1	28.5	170.7	21.3 bc	14 210
Rexelle	13.7	17.8	20.6	21.6	21.0	18.5	17.4	22.8	153.4	19.2 cd	15 340
Heimanns Rubinweichsel	14.5	16.6	18.9	20.8	20.5	17.5	18.3	18.6	145.7	18.2 cd	14 570
Kelleriis 16	8.2	13.6	18.7	19.2	18.5	17.1	15.6	20.3	131.2	16.4 cd	10 922
Heimanns Konservenweichsel	14.5	16.8	17.5	15.2	13.2	15.4	14.3	16.6	123.5	15.4 cd	10 281
Kelleriis 14	9.1	12.6	18.7	16.2	15.2	12.7	14.2	16.1	114.8	14.4 d	9 557
Schattenmorelle	9.7	12.2	15.4	17.2	15.2	11.8	16.2	15.5	113.2	14.2 d	9 424

Means followed by the same letter do not differ significantly according to Tukey's test at P=0.05

As opposed to our results, Stan evic (1969) in ecological conditions of a ak evaluated ‘Richmorency’, ‘Heimanns Rubinweichsel’ and ‘Schattenmorelle’ as very productive and productive cutivars. Nikolic et al. (2000), under similar ecological conditions, assessed as the most productive ‘Obla inska’, ‘Heimanns Konservenweichsel’, ‘Rexelle’, ‘Kelleriis 14’ and ‘Kelleriis 16’.

### Conclusion

Flowering of examined cherry cultivars in ecological conditions of Kraljevo, on average, occurs in the second decade of April, and lasted about 11 days. Early flowering was observed in cultivars ‘Richmorency’, ‘Obla inska’, ‘Heimanns Rubinweichsel’ and ‘ a anski Rubin’, followed by ‘Heimanns Konservenweichsel’, ‘Kelleriis 16’ and ‘Rexelle’, while late flowering was observed in ‘Schattenmorelle’ and ‘Kelleriis 14’. The difference in flowering time between cultivars was almost twice higher than that between years.

The time span between the cultivars of the earliest and the latest average fruit ripening was approximately 16 days, and the biggest difference between years was 22 days. ‘Richmorency’ and ‘Obla inska’ are characterized by middle-early ripening (25-30 June), followed by medium late ‘ a anski Rubin’, ‘Heimanns Rubinweichsel’, ‘Rexelle’, ‘Kelleriis 16’ and ‘Heimanns Konservenweichsel’ (1-2 July). ‘Schattenmorelle’ and ‘Kelleriis 14’ proved to be late maturing (8-9 July).

The average annual yield per tree amounted to 14-28 kg. The highest cumulative yield per tree was recorded in 'Rexelle' (224 kg), and the lowest in 'Keleris 16' and 'Schattenmorelle' (113.2 kg). Productivity of sour cherry cultivars, in addition to their biological properties, depends to a large extent on the ecological conditions of the area where they are grown.

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**MORPHOLOGICAL AND PRODUCTION CHARACTERISTICS OF SPELT  
WHEAT ON THE CHERNOZEM AND DEGRADED SOIL**

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**Abstract**

The effect of agro-ecological conditions on morphological and production characteristics of the first domestic spelt wheat cultivar - *Nirvana* was studied. *Nirvana* was selected by the Institute of Field and Vegetable Crops from Novi Sad. It is a winter cultivar, very tolerant to frost. In the period 2011-2013, field micro experiments were set up on two locations - on an experimental field of the "Tamis" Institute on a carbonated (micellar) chernozem soil formed on the loess terrace, and on a field in the vicinity of the Thermal Power Plants "Nikola Tesla" on a degraded soil, formed on a coal-mine dumping ground and uncultivated for more than 15 years. The agro-ecological conditions on these two locations affected the growth and productivity of the spelt wheat. Although average weather conditions on both locations were relatively favourable, growth and development were more intensive in the second year of research. The effect of soil conditions on morphological characteristics was highly statistically significant. The plants grown on the chernozem had 45% higher stems, 46% longer spikes and about 6.4% more spikelets per spike. The quality of soil also had significant effect on yield indicators, so the plants grown on the chernozem had 16.23% more grains per spike and 19.8% larger grain weight per spike. The two-year average yield of dehulled grains obtained on the chernozem was 3010 kg ha<sup>-1</sup>, 35% higher than the yield obtained on the degraded soil. Despite all the indicators of the plants grown on the degraded soil were significantly lower, yet it can be concluded this type of wheat achieved satisfying grain yield. **Key words:** spelt wheat, morphological and production characteristics, yield data, soil type.

**Introduction**

Spelt wheat is a species that belongs to the one of the most ancient genus of cereals, *Triticum*. Spelt wheat was known and cultivated in ancient Egypt 6000 years ago. Ancient Romans used spelt wheat grain in their diet, expanding its production across the Roman Empire – today's Europe and northern Africa. The production of spelt wheat retained in the mountainous regions of south-eastern Europe until the 20th century (*Glamoclija et al.*, 2012a, 2012b). The growing of spelt wheat has recently become very popular, especially after studying the relationship among the plants and agro-ecological and soil conditions, and finding the most appropriate production technology (*Stallknecht et al.*, 1996). After studying the chemical characteristics of spelt wheat grain and flour, *Zielinski et al.* (2008) point out that this cereal is great for making bread of a higher nutritional value. Special bread and pastry products are very well accepted, especially in countries in which growing number of population increasingly use organic products (*Abdel-Aal et al.*, 1995). Growing this cereal is also becoming more interesting in Serbia. Areas under spelt wheat are increasing in hilly-mountainous but also in lowland regions.

The objective of this research is to study basic production characteristics of the first domestic spelt wheat cultivar *Nirvana*, grown on the most fertile soil in Serbia and on degraded soil that is being used for field crop production after 15 years.

### Material and Methods

In the period 2011-2013 field micro experiments were set up on two locations, on an experimental field of the "Tamis" Institute in Pancevo and on a field in the vicinity of "TENT 2" - Thermal Power Plants "Nikola Tesla" in Obrenovac (Usce). The experimental field in Pancevo was on a carbonated (micellar) chernozem soil formed on the loess terrace. Because of its natural fertility, a favourable soil solution reaction and physical characteristics, this soil type has a huge potential for wheat production (*Glamoclija et al.*, 2012a, 2012b). Another experimental field was on a degraded soil, formed on a coal-mine dumping ground and uncultivated for more than 15 years. The subject matter of this research was the first domestic spelt wheat cultivar *Nirvana*, selected by the Institute of Field and Vegetable Crops from Novi Sad. This cultivar is a winter crop and it is very frost-tolerant. According to the results of previous research (*Mladenovic & Dencic*, 2010), *Nirvana* spelt wheat cultivar can be successfully grown on less fertile soils and it is more resistant to draught than common wheat. Its requirements for nitrogen are lower, so the best results this cultivar achieves on moderately fertile soils, while under conditions of much fertile soils and in intensive nitrogen fertilization it is prone to lodging due to its high stem. Cropping potential of *Nirvana* is over 4000 kg ha<sup>-1</sup>. On the top of the stem (average height 107 cm) is a spike with average length of 10-15 cm, with about 20 two-blossom spikelets. During threshing, the grain remains tightly wrapped in glumes, lemmas and paleas. Dehulled grain volume weight is 75-78 kg and 1000-grain weight is about 41 g. Grains contain up to 17.5% of total proteins. Due to a specific ratio of gliadine and glutenin, spelt wheat flour can be used for making special pastry products of a high nutritional value, rich in vitamins B and mineral salts. When compared to common wheat, spelt wheat grains contain 7 to 8 times more calcium, magnesium, phosphorus and selenium. Mineral salts in grains are bound to organic carbon and easily absorbed by the organism. Due to its biological characteristic, *Nirvana* is suitable for organic production.

On the experimental field of the "Tamis" Institute, standard cropping practices for common wheat (*Triticum vulgare* L.) are applied. A pre-crop was sunflower. The basic post-harvest tillage was conducted, when 250 kg ha<sup>-1</sup> of NPK fertilizer 15:15:15 was applied. Considering spelt wheat small requirements for nitrogen and its susceptibility to lodging, no top-dressing nitrogen was applied on the crops. At the same time, pre-sowing preparation was performed at the depth of 6-8 cm. Sowing was done with an experimental sowing machine in late October. During the growing season, no crop tending or plant protection measures were applied. Prior to harvest, conducted with an experimental combine in early July, samples for testing the following parameters were taken: stem height, spike length, number of spikelets, number of grains, grain weight and chaff weight.

At the experimental field in the vicinity of "TENT 2" - Thermal Power Plants "Nikola Tesla" in Obrenovac after amelioration (cleaning the plot of different waste and removing weeds), in summer the soil was ploughed down to 20 cm of depth, and in autumn it was prepared for sowing. In pre-sowing fertilization, 600 kg ha<sup>-1</sup> of NPK mineral fertilizers 15:15:15 were used. Sowing was done manually in late October. During the growing season no crop tending or plant protection measures were applied. Harvest was done manually in early July and samples for testing the following parameters were taken: stem height, spike length, number of spikelets, and number of grains, grain weight and lemma/palea weight.

All data were then processed using the analysis of variance and LSD test.

**Growing conditions**

During the research, the basic meteorological data for both experimental fields were monitored. Data on monthly rainfall and air temperatures in 2011/12 and 2012/13 were taken from the Republic Hydro-Meteorological Service of Serbia.

**Rainfall.** Total rainfall sums during spelt wheat growing season at both experimental fields were smaller in the first year of the research (Table 1).

Table 1. Rainfall sums during spelt wheat growing season, mm

Month	P a n c e v o			O b r e n o v a c		
	2011/12	2012/13	Average	2011/12	2012/13	Average
X	39	38	50	45	49	50
XI	51	71	51	57	45	55
XII	55	30	59	54	61	59
I	45	73	40	45	82	45
II	40	46	35	41	62	35
III	42	1	37	48	3	44
IV	57	80	55	56	77	55
V	52	100	47	53	128	56
VI	85	10	80	88	19	82
VII	16	67	65	25	24	63
	<b>437</b>	<b>516</b>	<b>517</b>	<b>512</b>	<b>550</b>	<b>537</b>

In 2011/12 in Pancevo there was 18% and in Obrenovac about 5% less rainfall compared to the multiannual average. Despite smaller total sums, rainfall distribution was favourable. In months of the highest water consumption (April, May, first half of June), the water regime at both locations was favourable. In the second year, Pancevo rainfall sums for spelt wheat growing season was in line with the multiannual average, while rainfall sums in Obrenovac were 2.5% higher than the average. Monthly water regime was very favourable, with the highest values in the second half of April and in May, that is, in the period of the highest water consumption.

**Temperature conditions.** In the first year, the average air temperatures for spelt wheat growing period were 0.7-0.8<sup>0</sup>C higher on both locations (Table 2).

Table 2. Air temperature during growing period of spelt wheat, <sup>0</sup>C

Month	P a n c e v o			O b r e n o v a c		
	2011/12	2012/13	Average	2011/12	2012/13	Average
X	13	11	13	12	10	11
XI	5	6	7	5	6	7
XII	6	2	2	4	3	4
I	3	-0.3	1	3	0	2
II	-3	2	3	-2	2	3
III	10	6	7	11	7	8
IV	14	13	13	14	14	15
V	18	18	18	17	18	18
VI	25	21	21	24	22	20
VII	26	23	24	29	23	22
Average	<b>11.7</b>	<b>10.2</b>	<b>10.9</b>	<b>11.7</b>	<b>10.5</b>	<b>11.0</b>

In the second year, the average air temperatures on both locations were 0.5-0.7<sup>0</sup>C lower than the average for this region.

The analysis on the monthly heat distribution in the first year showed that the winter was extremely cold with a long period of frosts. Since the crops were covered by snow, those frosts did not affect the spelt wheat. On the other hand, summer months were very hot. In the winter of second year there were no long periods of frosts, and the summer was characterized by more favourable air temperatures for the generative development of the crops, which had a positive effect on grain filling and synthesising of grain nutrients.

### Results and Discussion

**Stem height.** The average stem height at the time of harvest was 96.8 cm on both experimental fields. The crops grown on the chernozem had considerably higher stems (114.6 cm) than the crops grown on the degraded soil (79.0 cm), in the two-year average and by years of variation (Table 2). Between-year variations in stem height were also statistically significant. In 2013, higher stems were formed on both locations. Although this species is less susceptible to adverse soil conditions than common wheat, crops need optimum agro-ecological and soil conditions for their growth, as *Stallknecht and Gilbertson* (1995) pointed out.

**Spike length.** Spike length was 10.99 cm in the two-year average. Variations were significant by the locations and years. The longest spikes were formed in the second year by the crops grown on the chernozem soil in Pancevo, while the shortest spikes (8.63 cm) were formed in the first year by the crops grown the degraded soil of the thermal power plant. Some previous research on the effects of agro-ecological conditions on morphological and production characteristics of spelt wheat have shown that spike formation strongly depends on weather and soil conditions (*Ugrenovic et al.* 2012).

**Number of spikelets per spike.** The average number of spikelets per spike varied from 18.87 (degraded soil, the thermal power plant, 2012) to 21.28 (chernozem, Pancevo, 2013). Total variation in number of spikelets per spike was significant both by the locations and years.

**Number of grains per spike.** In the total average, the number of grains per spike was 28.05. Comparing this value with the number of spikelets per spike, it can be concluded that the spikelets on average had 1.4 grain. The number of grains per spike was significantly affected by soil conditions. The crops grown on the degraded soil formed spikes with 25.94 grains, while the crops on the chernozem had 30.15 grains. This difference, together with the differences between the years, was statistically significant to very significant.

**Grain weight per spike.** The crops grown on the chernozem formed longer spikes with more spikelets and grains of 1.27 g of weight in the two-year average. This value is about 20% higher, compared with the one in the crops grown on the degraded soil. Group and individual variations of grain weight per spike were statistically significant. The crops grown on the chernozem formed higher stems and longer spikes with more spikelets per spike, more grain per spike and larger grain weight per spike.

**Dehulled grain yield.** This value shows a spelt wheat yield after dehulling the grains using specialized hullers. The average two-year grain yield for the whole experiment was 2620 kg ha<sup>-1</sup>. The yield on the chernozem varied from 2850 kg ha<sup>-1</sup> (2012) to 3170 kg ha<sup>-1</sup> (2013). The average grain yield on the degraded soil in the first and second year was 35% and 42% lower than the one on the chernozem. This variation together with the variations between the years was statistically significant.

However, although the difference in yields was statistically significant, it should be pointed out that the degraded soil gave higher grain yield, which implies that this species can be grown in adverse edaphic conditions (*Glamoclija et al.*, 2010).

Table 3 Indicators of morphological and production characteristics

Characteristics, Location, Year	Stem height	Spike length	Number of spikelets per spike	Number of grains per spike	Grain weight per spike,g	Grain yield, kg ha <sup>-1</sup>
PPS, 2012	107.4	12.55	19.58	29.12	1.06	2850
PPS, 2013	121.7	13.53	21.28	31.17	1.48	3170
Average	114.6	13.04	20.43	30.15	1.27	3010
TENT, 2012	77.7	8.63	18.87	24.33	1.03	2107
TENT, 2013	80.3	9.23	19.55	27.55	1.09	2350
Average	79.0	8.93	19.21	25.94	1.06	2229
<b>Total average</b>	<b>96.8</b>	<b>10.99</b>	<b>19.82</b>	<b>28.05</b>	<b>1.17</b>	<b>2620</b>
LSD	0,05 8.14;	0,05 0.981;	0,05 0.411;	0,05 1.211;	0,05 0.53;	0,05 925.1;
2011/12	0,01 12.33	0,01 1.484	0,01 0.621	0,01 1.821	0,01 0.806	0,01 1397.8
LSD	0,05 18.7;	0,05 2.081;	0,05 1.741;	0,05 2.421;	0,05 0.352;	0,05 629.9;
2012/13	0,01 27.2	0,01 2.965	0,01 2.542	0,01 3.632	0,01 0.510	0,01 907.8

PPS - "Tamis" Institute, Pancevo; TENT - TPP "Nikola Tesla", Obrenovac

### Conclusions

Considering the results of the research on the effect of soil conditions on morphological and productive characteristics of spelt wheat, the following can be concluded:

Despite of the relatively favourable average weather conditions on both locations, spelt wheat growth and development were more intensive in the second year of the research;

The effect of soil conditions on morphological indicators was statistically very significant. The crops grown on the chernozem formed 45% higher stems, 46% longer spikes and about 6.4% more spikelets per spike;

The quality of soil significantly affected yield indicators. The crops grown on the chernozem formed 16.23% more grains per spike and had 19.8% larger grain weight per spike;

The two-year average yield of dehulled grains grown on the chernozem was 3010 kg ha<sup>-1</sup>, 35% higher than the yield on the degraded soil;

Despite all the indicators of morphological and production characteristics of the spelt wheat grown on the degraded soil of the Thermal Power Plants "Nikola Tesla" in Obrenovac were significantly lower than the one of the crops grown on the chernozem, it can be concluded that this species achieved satisfactory grain yield.

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**THE INFLUENCE OF METEOROLOGICAL PARAMETERS ON FRUIT  
DOUBLING IN STONE FRUIT SPECIES**

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**Abstract**

Exposure of stone fruit species to high temperatures during flower-bud differentiation leads to the occurrence of double pistils, which results in the development of double fruits. In order to analyze this phenomenon in our ecological conditions, tests were carried out during the three-year period (2011-2013) in the collection orchards of the Experimental farm “Radmilovac” of the Faculty of Agriculture in Belgrade. The influence of air temperature and rainfall on fruit doubling was studied in 16 cultivars of peaches and nectarines, 15 cultivars of apricot and 10 cultivars of plum.

The average percentage of double fruits in a three-year period was the highest in peach (20.0%), followed by plum (10.9%) and apricot (4.1%). In peach and nectarine cultivars the average percentage of double fruits was the highest in 2013 (38.8%), and it was more than three times higher comparing with other two years. In plum cultivars the average percentage of double fruits was also the highest in 2013 (13.7%). In apricot cultivars the percentage of double fruits was the lowest in 2013 due to very low fruit set because of unfavorable weather conditions for pollination.

**Key words:** peach, plum, apricot, double fruits, temperature, rainfall.

**Introduction**

Double fruits or ‘twins’ occur in some stone fruits species (peach, plum, cherry, apricot) in many production regions with arid climates (Kudela and Krejzar, 2005). Fruit doubling occur when the flower buds are under stress at the initial phase of their differentiation. The lack of water and high temperatures affect the fruit doubling (Patten et al., 1981; Johnson et al., 1992; Naor et al., 2005). Double-fruits have no market value and should be removed by thinning.

Handley and Johnson (2000) found that the fruit doubling in peach can be significantly reduced by irrigation shortly before or during carpel differentiation (late August in California). Setting up nets above the orchard that shading the trees can also contribute to reducing the incidence of double fruits (Beppu and Kataoka, 2000).

Cultivar is the most important factor in production of fruits (Ogašanovic et al., 2005). Cultivars of stone fruits differ in the extent of fruit doubling. Engin and Ünal (2004) reported that the occurrence of double fruits is especially pronounced in peach cultivars ‘Cardinal’ and ‘Dixired’.

The aim of this study was to investigate the occurrence of double fruits in ecological conditions of the region of Belgrade in a number of cultivars of peach, nectarine, plum and apricot.

### Material and methods

The tests were performed in collection orchards of the Experimental farm “Radmilovac” of the Faculty of Agriculture in Belgrade. Studies were conducted on 16 cultivars of peach and nectarine, 15 cultivars of apricot and 10 plum cultivars. Most of the tested cultivars are significantly represented in fruit production in Serbia. The tests were performed in young plantations, whose age was 3-7 years, and which are grown without irrigation. Peach orchard is maintained as high density system, with spacing 3.6 x 1.3 m, and trees are trained as Sloping Leader (Zec et al., 2013). Plum orchard has spacing of 4 x 3 m and training system is Central Leader. Apricot orchard was established with space 4,5 x 3 m and training system is Central Leader.

Percentage of double fruits was measured on tagged shoots containing approximately 500 flowers. Number of fruits was counted 1 month after bloom. The study was conducted during the three-year period (2011-2013). The most important meteorological parameters were monitored using automatic weather station “Metos” during the initial period of differentiation of flower buds (July to September) and they are presented in Table 1.

Table 1. The average monthly temperatures and total rainfall in the period July-September at the Experimental farm „Radmilovac“ (2010-2012)

Month	2010		2011		2012	
	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)
July	22.8	44	22.4	70	25.1	22
August	22.0	27	22.7	6	24.1	4
September	16.9	56	20.7	42	20.0	18
Average / Sum	20.6	127	21.9	118	23.1	44

Data were statistically analyzed using analysis of variance for two-factorial experiment. The significance of differences between mean values was determined using LSD test for the probability of 0.05.

### Results and discussion

Fruit doubling is occurring every year in peaches and nectarines in varying degree. During the period of study, the highest average percentage of double fruits in all cultivars was obtained in 2013 and it was 38.8% (Table 2). The lowest percentage of double fruits was found in 2011 (10.2%). Mean values of double fruits percentage in peaches and nectarines were significantly higher in 2013 compared with two other years.

The average air temperature in the period July - September of 2012 was 1.2°C and 2.5°C higher than in the same period in 2011 and 2010, respectively. In the same period in 2012 fell nearly 3 times less rainfall compared with two other years (2010 and 2011). In the period July - September of 2012, fruit trees were exposed to severe stress due to high temperatures and drought, resulting in the appearance of a high percentage of double fruits in the next year (2013) in all cultivars of peach and nectarine. Naor (2006) also noted that high temperature and drought stress in July and August initiate the appearance of double pistils in peach.

Table 2. Average values of double fruits occurrence in peach and nectarine cultivars (%)

Cultivar	Year			Average
	2011	2012	2013	
Autumn Glo	17.67	20.33	68.67	35.6 g
Big Top	2.67	2.00	30.33	11.7 b
Bolero	3.67	3.00	29.33	12.0 b
Caldesi 2000	14.00	12.00	34.00	20.0 c
Maria Lucia	9.33	12.00	32.00	17.8 c
Max 7	11.00	15.00	42.00	22.7 d
Morsiani 51	16.33	13.67	56.67	28.9 e
Orion	15.00	12.00	44.33	23.8 d
Redhaven	8.33	9.00	24.00	13.8 b
Rita Star	5.67	4.67	15.00	8.4 a
Royal Glory	10.00	12.33	33.33	18.6 c
Sirio	10.00	14.33	60.67	28.3 e
Sun Prince	7.67	8.67	23.33	13.2 b
Venus	10.00	8.00	22.67	13.6 b
Vin anka	14.33	21.00	63.00	32.8 f
White Glo	7.00	8.33	41.67	19.0 c
Average	10.17 a	11.02 a	38.81 b	20.00
LSD 0.05	Cultivar			2.30
	Year			0.90

The highest percentage of double fruits in the period of study (2011-2013) was recorded in 'Autumn Glo' peach (35.6%), and the lowest percentage was found in nectarine 'Rita Star' (8.4%). Differences between cultivars were statistically significant. Perez-Gonzalez and Merlin (2001) state that later blooming cultivars are more prone to fruit doubling, and that this trait is influenced by genotype.

The highest percentage of double fruits (13.7%) in plum cultivars was found in 2013 (Table 3). The lowest percentage of double fruits was recorded in 2011 (8.4%). Mean values of fruit doubling percentages in plum significantly differed between years of study.

Table 3. Average values of double fruits occurrence in plum cultivars (%)

Cultivar	Year			Average
	2011	2012	2013	
a anska lepotica	9.33	12.67	8.00	10.0 d
a anska najbolja	9.00	11.33	12.33	10.9 d
a anska rana	14.33	18.00	16.67	16.3 f
a anska rodna	5.00	3.67	5.33	4.7 b
Delikatnaja	3.33	2.67	17.33	7.8 c
Elena	10.00	20.33	33.00	21.1 g
Grossa di Felicio	0.00	0.00	0.00	0.0 a
Jojo	7.67	7.00	14.67	9.8 d
Stanley	13.33	15.00	16.00	14.8 e
Toper	11.67	16.00	13.67	13.8 e
Average	8.37 a	10.67 b	13.70 c	10.91
LSD 0.05	Cultivar			1.11
	Year			0.61

Extreme weather conditions (high temperatures and drought) in the summer of 2012, contributed to increased occurrence of double fruits of plum cultivars in the 2013, such as in peaches. Kudela and Krejzar (2005) also reported increased occurrence of double fruits in plum as a result of exposure to high temperatures and drought during the previous summer.

The highest percentage of double fruits in the three-year period was obtained in cultivar ‘Elena’ (21.1%), and the lowest percentage was found in cultivar ‘Grossa di Felicio’ (0%). The obtained differences are genetically determined.

The studied apricot cultivars had a lower percentage of double fruits in comparison with peaches, nectarines and plums. For 2011 and 2012 years it was averaged 6.1% (Table 4). In 2013 double fruits were not found in apricot cultivars. In this year the weather conditions during the flowering of apricots (late March and early April) were very unfavorable. Low daily temperatures (below 10°C) and the absence of bees resulted in very poor fruit set and low yield.

Table 3. Average values of double fruits occurrence in apricot cultivars (%)

Cultivar	Year			Average
	2011	2012	2013	
Cegledi Arany	8.00	6.67	0.00	4.9 d
Goldrich	11.00	1.67	0.00	4.2 bcd
Hungarian Best	7.67	14.67	0.00	7.4 e
Laycot	1.67	2.33	0.00	1.3 a
Lenova	4.67	1.67	0.00	2.1 a
Leskora	8.00	3.00	0.00	3.7 bc
Litoral	8.00	2.67	0.00	3.6 bc
Neptun	7.33	18.67	0.00	8.7 g
Ninfa	2.67	1.67	0.00	1.4 a
Novosadska rodna	10.33	2.00	0.00	4.1 bcd
Pisana	2.33	4.33	0.00	2.2 a
Re Umberto	2.33	12.67	0.00	5.0 d
Silred	7.33	2.67	0.00	3.3 b
Silvercot	10.33	2.67	0.00	4.3 bcd
Sundrop	11.00	2.33	0.00	4.4 cd
Average	6.84 a	5.31 b	0.00 c	4.05
LSD 0.05	Cultivar			0.90
	Year			0.40

Obtained results for 2011 and 2012 years also indicated that tested apricot cultivars significantly differed in percentage of double fruits. Cultivars ‘Neptun’ and ‘Hungarian Best’ were the most susceptible to fruit doubling. The results obtained in this study are consistent with the findings of Milatovic and Stojanovic (2005). They report that the occurrence of double pistils in apricot is relatively small and that only in some cultivars and in some years it exceeds 10%.

### Conclusion

High air temperatures and low amount of rainfall affect the significant increase of fruit doubling in cultivars of peach, nectarine and plum.

It was noted that some cultivars of peach, nectarine and plum have a genetic predisposition for a higher percentage of double fruits.

Cultivars that are more prone to the formation of double fruits should be grown under irrigation. It could reduce the effects of adverse weather conditions on the intensity of this phenomenon.

Occurrence of double fruits in tested cultivars of apricot was expressed to a lesser extent compared with peaches, nectarines and plums. To define apricot cultivars precisely in terms of this trait it is necessary to continue the research.

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**EFFECT OF SHOOT HEADING DATE ON SYLLEPSIS AND SYLLEPTIC SHOOT TRAITS IN PLUM ČAČANSKA LEPOTICA**

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**Abstract**

Apart from frequent spontaneous syllepsis, sylleptic shoot development in plums can be induced by diverse practices, most notably plant hormone application and shoot tip removal. Research was conducted in 2008 and 2009 to evaluate the effect of summer pruning heading date on the degree of sylleptic branching and major morphological and anatomical properties of sylleptic shoots in plum 'čačanska Lepotica' grafted on Myrobalan (*Prunus cerasifera* Ehrh.) seedling rootstock. Shoots were cut back to 4-5 buds above the base at 5 dates (D<sub>1</sub> – 20 May, D<sub>2</sub> – 5 June, D<sub>3</sub> – 20 June, D<sub>4</sub> – 5 July and D<sub>5</sub> – 20 July). At the end of dormancy, sylleptic shoots were subjected to morphological measurement: sylleptic shoot length and diameter (cm), number of nodes, internode length (cm), number of vegetative buds, number of flower buds, and anatomical analysis: primary xylem length (µm), number of tracheae per mm<sup>2</sup> and trachea width (µm). Results showed that at the late heading dates (D<sub>4</sub> and D<sub>5</sub>) sylleptic branching was absent in a large percentage of shoots (81.59% at D<sub>4</sub> and 94.10% at D<sub>5</sub>). In contrast, the highest positive response was observed for dates D<sub>2</sub> and D<sub>3</sub> which led to sylleptic shoots reaching moderate length (L<sub>2</sub> = 52.79 cm, L<sub>3</sub> = 22.09 cm), with a very good vegetative to flower buds ratio (at D<sub>2</sub> - 1:0.43, and at D<sub>3</sub> 1:0.98). The sylleptic shoots emerging at dates D<sub>2</sub> and D<sub>3</sub> had the following anatomical properties: primary xylem width 94.79 µm and 70.43 µm; number of tracheae per mm<sup>2</sup> 141.18 and 134.88, and trachea width 3.09 µm and 3.07 µm, respectively. Data suggest that 5-20 June, or D<sub>2</sub> and D<sub>3</sub> as used in this study, is the most suitable date to cut back shoots in plum 'čačanska Lepotica' for sylleptic branching.

**Keywords:** plum, sylleptic shoots, morphological and anatomical properties, shoot heading.

**Introduction**

Sylleptic shoot formation in fruit trees is affected by a variety of factors, primarily fruit species and cultivar (Wertheim 1978; Marini 2010). Some fruit crops, such as peaches [*Prunus persica* (L.) Batsch], exhibit an increased genetic tendency for the development of sylleptic shoots compared with other fruit trees (Hipps et al., 1995). The use of some agricultural practices such as an intensified supply of mineral fertilisers, particularly nitrogen, can promote not only proleptic but also sylleptic shoot formation (Jordan et al. 2009). In general, any practice or technique that contributes to fruit tree vigour and growth – irrigation, more severe pruning and nitrogen supply – enhances sylleptic branching (Chalmers et al., 1981; Jordan et al., 2009). Nonetheless, key stimulators of sylleptic shoot formation include plant hormones (cytokinins and auxins) (Cook et al., 1998; Cline and Dong-II, 2002), and mechanical injury or pruning during the growing season (Oullette and Young, 1994).

Sylleptic branching in different fruit crops can have both positive and negative aspects. The occurrence of sylleptic i.e. feather shoots in nursery trees is positive and advantageous (De

Wit et al., 2002). On the other hand, in young fruit trees during the second or third year after planting, extensive sylleptic shoot development can cause tree training problems, thus revealing its negative aspects. In some cases, sylleptic branching may significantly increase leaf area and the general growth of the tree (Cline and Dong-Il, 2002). Also, in some fruit trees that have a thin crown and a tendency to produce blind wood, such as plums, sylleptic shoot development is highly desirable.

In modern plum orchards under High Density Planting system (HDP), summer pruning is a mandatory practice (Milosevic et al., 2008) which, inter alia, promotes sylleptic branching and prevents blind wood and the movement of the bearing potential and fruiting zone towards the top and periphery of the crown (Milosevic et al., 2009). Serbian plum cultivar ‘ a anka Lepotica’ has the aforementioned negative tree properties (Nenadovic-Mratinic et al., 2007), particularly under HDP system.

The main objective of this study was to determine an optimal timing for summer pruning heading cuts in cv. ‘ a anka Lepotica’ trees during the first part of the growing season to stimulate the development of sylleptic shoots that would exhibit normal morphological traits. In addition, the anatomy of sylleptic shoots was studied. This allowed a broad analysis of both morphological and anatomical characteristics, thus contributing towards a good understanding of sylleptic shoot formation in plum trees.

## Materials and methods

### *Plant material and field trial*

Research was conducted from 2008 to 2009 to evaluate Serbian plum cultivar ‘ a anka Lepotica’ budded onto Myrobalan seedling rootstock at 25 cm above ground level. The orchard was established in 2003. The choice of cultivar was due to the potential interest in this cultivar in the a ak region, because of its maturity time and good fruit quality.

The experimental orchard was established at Gornja Gorevnica near a ak (43°53’N latitude; 20°21’E longitude; 390 m a.s.l.), Western Serbia, five and six years after planting. Trees were planted under HDP system at a spacing of 4 m × 2 m (1,250 trees ha<sup>-1</sup>) and trained to the Spindle Bush system. Orchard management was consistent with standard practices for HDP, except irrigation. Summer pruning was used. In addition, climatic conditions were similar in both years.

### *Experimental procedure and analysis of the morphology and anatomy of sylleptic shoots*

Heading shoots to four buds during the growing season was conducted at 5 dates (T). The first heading date (T<sub>1</sub>) was 30 days after the onset of shoot growth (20 May), the remaining four at 15-day intervals: T<sub>2</sub> = 5 June, T<sub>3</sub> = 20 June, T<sub>4</sub> = 5 July and T<sub>5</sub> = 20 July. Each heading treatment involved the cutting back of 20 shoots in four replications (totalling 80 shoots) and subsequent monitoring of sylleptic shoot development.

Sylleptic shoots were collected for morphological and anatomical analyses in the first ten days of February in the following year. The morphological traits analysed included: sylleptic shoot length and diameter (cm), number of nodes, internode length (cm), number of vegetative buds and number of flower buds per shoot. A ruler and a digital caliper (Starrett, 727 Series, Athol, New England, USA) were used. The shoots were subjected to the following anatomical measurement: primary xylem width (µm), number of tracheae per mm<sup>2</sup> and trachea width (µm). The specimens collected for anatomical analysis were sectioned using a Reichert, Biocut 2030 sliding microtome (Germany). Permanent histological mounts were prepared by standard procedure. Thereafter, a microscope (Reichert, Germany) was used to measure tissue parameters. Primary xylem width and trachea width were measured under 50x



and 400x magnification, respectively, and tracheae number per mm<sup>2</sup> were counted under 100× magnification. Images of the cross-sections of sylleptic shoots were taken with a Leica DC 300 camera, and processed and analysed by the Leica IM 1000 software. The terminology of wood anatomical aspects followed Wheeler et al. (1989).

#### Data analysis

The data obtained were analysed according to a factorial design arranged in a randomised complete block design with four replicates, with heading dates and years as factors, each with three and/or two levels (dates: T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub>; years: 2008 and 2009). Analysis of variance (ANOVA) was performed at  $P \leq 0.05$  and  $P \leq 0.01$  significance levels, followed by an LSD test at  $P \leq 0.05$  and  $P \leq 0.01$  using the MSTAT-C statistical package (Michigan State University, East Lansing, MI, USA).

### Results and discussion

Results on the response of ' a anska Lepotica' shoots to heading treatments during the growing season are presented in Table 1.

Tab. 1. Sylleptic shoot development in ' a anska Lepotica' depending on heading date

Response	No sylleptic branching (%)	Vegetative sylleptic shoots (%)	Generative sylleptic shoots (%)	
Heading date ( )				
1	3.56±0.18 d	74.02±6.47 a	22.41±1.29 c	
2	5.56±0.39 d	64.85±4.11 b	29.59±2.02 b	
3	17.56±0.95 c	44.60±3.15 c	37.84±2.32 a	
4	81.59±6.15 b	13.00±0.84 d	5.41±0.36 d	
5	94.10±7.35 a	2.02±0.11 e	3.37±0.30 d	
Year (B)				
2008	39.93±3.07	39.61±2.83	20.45±1.21	
2009	41.01±2.94	39.79±3.01	19.20±1.31	
A × B				
1	2008	3.20±0.15	72.15±5.60	24.65±1.18
	2009	3.92±0.20	75.90±7.34	20.18±1.40
2	2008	5.52±0.38	65.30±4.33	29.18±2.20
	2009	5.60±0.40	64.40±3.90	30.00±1.85
3	2008	17.22±0.80	45.30±3.20	37.48±2.10
	2009	17.90±1.11	43.90±2.90	38.20±2.55
4	2008	80.85±5.90	13.20±0.90	5.95±0.33
	2009	82.33±6.40	12.80±0.78	4.87±0.40
5	2008	92.90±8.11	2.10±0.12	5.00±0.24
	2009	95.30±6.60	1.95±0.11	2.75±0.35
ANOVA				
	**	**	**	
B	ns	ns	ns	
A × B	ns	ns	ns	

Differences between the years were not significant, but those across heading dates were highly significant. At heading dates <sub>4</sub> and <sub>5</sub>, the absence of sylleptic branching was observed in a large percentage of shoots (81.59% at <sub>4</sub> and 94.10% at <sub>5</sub>). In contrast, the

percent of positive response was very high at heading dates  $T_1$ ,  $T_2$  and  $T_3$ , with sylleptic shoots developing in 82.44% to 96.44% of shoots headed at  $T_3$  and  $T_1$ , respectively.

Sylleptic shoots were mostly vegetative, and their percent decreased from  $T_1$  to  $T_3$ .

The vegetative activity of plum trees significantly declines in July, with shoot elongation ceasing (Bulatovic and Mratinic, 1996). Given that heading treatments at  $T_4$  and  $T_5$  occurred in July, the lack of response to these treatments can, in our opinion, be attributed to the above observation.

Results on the effect of shoot heading date on the morphological characteristics of sylleptic shoots in plum ' a anaska Lepotica' are given in Tab. 2.

Tab. 2. Morphological characteristics of sylleptic shoots in plum ' a anaska Lepotica' depending on shoot heading date

Parameter		Sylleptic shoot length (cm)	Sylleptic shoot diameter (mm)	Number of nodes	Internode length (cm)	Number of vegetative buds	Number of flower buds
Heading date (A) *							
	1	64.84±4.86 a	4.40±0.21 a	16.41±1.15 a	4.04±0.20	16.05±0.95 a	4.85±0.38 c
	2	52.79±4.56 b	4.07±0.25 a	15.56±1.20 a	3.84±0.20	15.37±1.00 a	6.72±0.46 a
	3	22.09±2.02 c	2.98±0.12 b	6.08±0.37 b	3.92±0.20	5.40±0.41 b	5.31±0.34bc
Year (B)							
	2008	47.33±3.93	3.84±0.22	12.57±0.86	3.90±0.21	12.16±0.84	5.47±0.42
	2009	45.49±3.69	3.79±0.17	12.79±0.94	3.96±0.19	12.39±0.72	5.79±0.37
A × B							
	2008	65.39±4.43	4.41±0.22	16.51±1.20 a	3.98±0.18	15.80±1.01	4.80±0.43
1	2009	64.30±5.29	4.39±0.20	16.32±1.10 a	4.10±0.22	16.30±0.89	4.90±0.34
	2008	55.41±5.22	4.10±0.31	15.32±1.09 b	3.79±0.19	15.30±1.10	6.30±0.51
2	2009	50.18±3.90	4.04±0.20	15.80±1.30 b	3.89±0.21	15.45±0.90	7.15±0.41
	2008	22.20±2.15	3.02±0.13	5.90±0.31 c	3.94±0.25	5.40±0.43	5.30±0.33
3	2009	21.99±1.90	2.95±0.11	6.25±0.44 c	3.90±0.14	5.41±0.39	5.32±0.35
ANOVA							
		**	*	*	ns	*	*
B		ns	ns	ns	ns	ns	ns
A × B		ns	ns	**	ns	ns	ns

\* At heading dates  $T_4$  and  $T_5$ , the percent positive response i.e. percentage of shoots positively responding to heading during the growing season and exhibiting vegetative or generative growth was extremely low (Tab.1.); accordingly, these dates were considered unfavourable and, therefore, not included in Tabs. 2 and 3.

As regards the morphological characteristics of the sylleptic shoots, the effect of heading dates was significant or very significant, except for node length which was not significantly affected by either heading date or year as variance factors. The effect of year as a variance factor was random for the other morphological parameters as well.

The  $T_1$  date resulted in the highest values for sylleptic shoot length (64.84±4.86 cm) and diameter (4.40±0.21mm). The lowest values were obtained at  $T_3$  (22.09±2.02cm and 2.98±0.12mm, respectively). The differences were very significant. No significant differences were observed in the number of nodes between  $T_1$  and  $T_2$  (16.41±1.15 at  $T_1$ ; 15.56±1.20 at

T<sub>2</sub>), whereas T<sub>3</sub> led to significantly fewer nodes on sylleptic shoots –  $6.08 \pm 0.37$ . Internode length showed no statistically significant differences. The flower to vegetative buds ratio on sylleptic shoots was most favourable at T<sub>3</sub> (close to 1:1), followed in a decreasing order by T<sub>2</sub> (1:2.5), and T<sub>1</sub> (approximately 1:4). The observed differences were significant. Heading at T<sub>3</sub> gave the shortest sylleptic shoots that were predominantly generative. At T<sub>2</sub>, sylleptic shoots showed moderate vigour and were either vegetative or generative, as opposed to vigorous vegetative sylleptic shoots developing at T<sub>1</sub>.

Our results regarding sylleptic shoot vigour are in agreement with the findings of Morgas et al. (1998), who reported that early summer pruning can induce vigorous growth of sylleptic shoots. Sylleptic shoots that emerge as a result of early summer pruning, i.e. early heading cuts are usually more vigorous, while those that develop later in the growing season are generally less vigorous (De Wit et al., 2002). In the present study, later heading cuts as part of summer pruning induced shorter sylleptic shoots that had fewer nodes and shorter internodes, but a higher number of flower buds, which is in agreement with a previous study on plum (Mika and Piatkowski, 1989). Results on the effect of heading date on the anatomical characteristics of sylleptic shoots in plum ' a anaska Lepotica' are given in Tab. 3.

Tab. 3. Anatomical characteristics of sylleptic shoots in plum ' a anaska Lepotica' depending on heading date

Parameter	Xylem width ( $\mu\text{m}$ )	Tracheae number per $\text{mm}^2$	Trachea width ( $\mu\text{m}$ )	
Heading date (A)				
1	$103.45 \pm 4.46$ a	$147.32 \pm 6.36$	$3.13 \pm 0.06$	
2	$94.79 \pm 4.45$ b	$141.18 \pm 5.51$	$3.09 \pm 0.05$	
3	$70.43 \pm 3.38$ c	$134.88 \pm 6.83$	$3.07 \pm 0.05$	
Year (B)				
2008	$90.03 \pm 4.26$	$140.88 \pm 6.56$	$3.11 \pm 0.05$	
2009	$88.97 \pm 3.94$	$141.37 \pm 5.90$	$3.08 \pm 0.06$	
A $\times$ B				
1	2008	$103.70 \pm 5.04$	$147.78 \pm 6.06$	$3.21 \pm 0.07$ a
	2009	$103.21 \pm 3.89$	$146.86 \pm 6.63$	$3.09 \pm 0.05$ c
2	2008	$94.63 \pm 4.36$	$141.00 \pm 5.90$	$3.08 \pm 0.05$ cd
	2009	$94.61 \pm 4.55$	$140.36 \pm 5.11$	$3.14 \pm 0.05$ b
3	2008	$71.77 \pm 3.40$	$135.35 \pm 7.54$	$3.05 \pm 0.04$ d
	2009	$69.10 \pm 3.37$	$134.40 \pm 6.16$	$3.05 \pm 0.04$ d
ANOVA				
	*	ns	ns	
B	ns	ns	ns	
A $\times$ B	ns	ns	*	

The above results showed that summer pruning heading date and year had no significant effect on the number of tracheae and trachea width in sylleptic shoots. Significant differences were observed only in xylem width. Xylem width was highest at T<sub>1</sub> ( $103.45 \pm 4.46 \mu\text{m}$ ), and lowest at T<sub>3</sub> ( $70.43 \pm 3.38 \mu\text{m}$ ), which was positively correlated with sylleptic shoot diameter presented in Tab. 2.

### Conclusion

Shoot heading in plum cv. ' a anska Lepotica' in the July treatment (dates T<sub>4</sub> and T<sub>5</sub>) gave no response in the majority of cases i.e. led to no sylleptic shoot development until the end of the growing season in question. Therefore, these dates cannot be considered suitable for summer pruning in promoting sylleptic branching.

Heading date T<sub>1</sub> is also considered unsuitable as it gave too vigorous vegetative sylleptic shoots.

At T<sub>2</sub> and T<sub>3</sub> heading treatments conducted as part of summer pruning, moderately vigorous sylleptic shoots exhibiting a favourable flower to vegetative buds ratio developed until the end of the growing season. Therefore, dates T<sub>2</sub> and T<sub>3</sub> lasting from 5 to 20 June provide an optimal timing for the summer pruning of shoots for enhanced crown vigour and sylleptic branching.

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**THE COMPETITIVENESS OF AZOTOBACTER, PSEUDOMONAS AND BACILLUS APPLIED AS A MIXTURE INOCULUM IN RHIZOSPHERE OF FIVE MAIZE GENOTYPES ASSESSED BY GENOTYPING AND PHENOTYPING METHODS**

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**Abstract**

The rhizosphere contain a different compounds produced by the plant roots. The plant growth-promoting rhizobacteria (PGPR) can colonize plant root and promote plant growth and some of them can reduce the incidence of soil-borne diseases. PGPRs are beneficial for agriculture and often used as biocontrol or biofertilizer inoculants. However, the variation in bacterial colonization and survival within the rhizosphere (rhizosphere competence) can cause inconsistency of the field results. In this study, the mixture of the selected bacteria (*Azotobacter*, *Pseudomonas* and *Bacillus*) has been used as inoculum to assess the competitiveness and effects on plant growth and yield of different maize genotypes. Rep-PCR using (GTG)<sub>5</sub> primer for BOX elements were applied. Phenotypic and PGP traits as well as persistence of inoculated strains in the total number of bacteria have been evaluated. The obtained results assessed by a combination of genotyping and phenotyping methods showed that mixture of strains (*Azotobacter*, *Pseudomonas* PS2 and *Bacillus* Q7) had better competitiveness to indigenous bacteria in the rhizosphere of all maize genotypes. Inoculation increased the total number of microorganisms by 61%, the number of N<sub>2</sub>-fixing bacteria by 49% and the number of azotobacters by 5% compared to the non-inoculated control i.e. indigenous bacterial population. PGP traits of *Azotobacter*, *Pseudomonas* PS2 and *Bacillus* Q7 influenced a growth and quality of maize.

**Key words:** Plant growth-promoting rhizobacteria (PGPR); rhizosphere competence; *Azotobacter*; *Pseudomonas*; *Bacillus*.

**Introduction**

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria (including species of *Pseudomonas*, *Bacillus*, *Azotobacter*, *Serratia*, *Azospirillum*, *Klebsiella*, *Arthrobacter* and *Burkholderia*), that can improve the plant growth both directly and indirectly. PGPR can be found in the rhizosphere, at root surfaces and in association with roots. Some of them can provide plant with PGP substances synthesized by the bacteria such as plant growth regulators – indoleacetic acid, gibberellic acid, cytokinins and ethylene (Joseph et al., 2007). PGPR improve plant growth by facilitating the uptake of certain plant nutrients from the environment during asymbiotic N<sub>2</sub> fixation, phosphate solubilization from insoluble mineral compounds and production of siderophores. In the indirect promotion of plant growth, PGPR can prevent deleterious effects of phytopathogenic microorganisms by production of antibiotics (Jamali et al., 2009; Jošić et al., 2012 *a, b*) and cyanide (Flaishman et al., 1996). To use in plant production, bacterial strains with several PGP traits need to be able to survive and colonize in the rhizospheric soil (Cattelan et al., 1999). Successful association

between introduced bacterial strains and plants cannot always be reproduced under field conditions, even good results were achieved in *in vitro* conditions (Zhender et al., 1999). The rhizospheric competence of introduced microorganisms depends on many factors, including the indigenous microbial flora in the soil (composition and activity) and environmental factors (climate, weather conditions, soil characteristics, etc).

In this study, the mixture of the selected bacteria (*Azotobacter*, *Pseudomonas* and *Bacillus*) was used as inoculum to assess the competitiveness and effects on plant growth and yield of different maize genotypes.

### Material and Methods

**Bacterial isolation.** *Pseudomonas* isolates were tested for fluorescence on King B medium (KB). *Bacillus* isolates were collected on Nutrient agar (NA) plates after incubation of soil suspension at 80<sup>0</sup>C for 15 min. *Azotobacter* isolates were screened on solid Fjodorov medium (FA) (Anderson, 1965). Isolates from each maize genotype (20 colonies per bacterial group) were tested for PGP traits and enzymatic activity. Isolates with the same or similar phenotypic properties (more than 80%) were subjected to genotyping using (GTG)<sub>5</sub> primer.

**Enzymatic activities.** Celulase, pectinase and protease activity were estimated as described by Milagres et al. (1999). Gelatinase activity was detected by liquefied solid gelatin, urease activity observed by color change using urea agar base supplemented with urea and amylase by zones on starch agar plates (Jha et al., 2008).

**PGP traits.** PSB trait was tested on Pikovskaya agar with 0.5% tricalcium phosphate [Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>] by plating method. After 7 days of incubation, phosphate solubilization was verified by clear halo appearance around colonies (Pikovskaya et al., 1949). Siderophore production was estimated using the chrome azurol S (CAS) assay, described by Schwyn & Neilands (1987) and modified by Milagres et al. (1999). Bacteria were grown on one half of the plate containing KB, NA or FA medium and simultaneously the siderophore production was detected on the other half containing CAS–blue agar.

**PCR assay.** Total DNAs were isolated from bacterial isolates and strains using heat–lysed method. Bacterial colonies, grown on KB (*Pseudomonas*), NA (*Bacillus*) and ON (*Azotobacter*) for 48 h at 25<sup>0</sup>C, were suspended in 300 µl of distilled sterile water, boiled for 8 min. and incubated on ice for 5 min. The lysed suspensions were centrifuged for 3 min at 13000 rpm and supernatants were used in PCR reactions. PCR reactions were carried out in a 25 µl volumes using Green Taq Dream master mix (Thermo Scientific, Lithuania) with 1µl of template DNA and 0.1 µmol of (GTG)<sub>5</sub> primer. Amplifications were performed in Eppendorf MasterCycler personal (Germany) with the temperature profile: an initial denaturation at 95<sup>0</sup>C for 7 min followed by 35 cycles of a three–step PCR program (94<sup>0</sup>C for 1 min, 52<sup>0</sup>C for 1 min and 65<sup>0</sup>C for 8 min) and a final extension at 65<sup>0</sup>C for 16 min (de Bruijn, 1992). Amplified products were separated by electrophoresis for 2h at 5V/cm in 1,2% (w/v) agarose gel with GeneRuler DNA Ladder mix SM0331 (Thermo Scientific, Lithuania).

### Results and Discussion

Plant growth promoting rhizobacteria (PGPR) can influence plant growth by various mechanisms. Effects of PGPR strains in different crops were already demonstrated (Wu et al., 2005). Bacterial inoculants are able to increase plant growth, protect plants from disease, improve seedling emergence and responses to external stress factors (Lugtenberg et al., 2002). The present study was designed to assess the mixture of selected rhizospheric bacterial strains *Bacillus* Q7, *Pseudomonas* PS2 and three *Azotobacter* strains (NM5, NM8 and NM 14) for their competitiveness and effects on plant growth and yield of different maize genotypes. The

enzymatic activities and PGP traits were used for obtaining the specific phenotype patterns (tab. 1). Isolates from different maize genotypes showing similar phenotypic patterns (more than 7 traits) were included in genotypic analysis. Rep-PCR (BOX type) using (GTG)<sub>5</sub> primer was appropriate for amplification of DNA fragments which form different patterns within the groups of applied bacteria in the mixture. Specific (GTG)<sub>5</sub> patterns were used to evaluate the persistence of inoculated strains in the same bacterial genera and to estimate colonization within the rhizosphere (tab. 2).

Table 1. Plant growth promoting traits, enzymatic activities and BOX type of introduced *Bacillus*, *Pseudomonas* and *Azotobacter* strains

Bacterial strain	Enzymatic activities <sup>a</sup>						PGP trait		Phenotype pattern	BOX type (GTG) <sub>5</sub>
	Celulase	Pectinase	Protease	Gelatinase	Urease	Amylase	Phosphate solubilization ability <sup>b</sup>	Siderophore production <sup>c</sup>		
<i>Bacillus</i> Q7	-	-	-	+	-	-	-	-	BI	B-A1
<i>Pseudomonas</i> PS2	-	-	+	-	+	-	+++	++	PII	P-A2
<i>Azotobacter</i> NM5	-	-	-	±	-	-	-	-	AIII	A-A1
<i>Azotobacter</i> NM8	-	-	-	±	-	-	++	-	AIII	A-A2
<i>Azotobacter</i> NM14	+	-	-	±	-	+	+	-	AIV	A-A3

<sup>a</sup>Protease, gellatinase, cellulase, pectinase, urease and amylase activities were determined by plate assay (+) hydrolysis; (-) no hydrolysis.

<sup>b</sup>Efficacy of phosphate solubilization evaluated according to halo diameter and colony diameter: (+) 1-4 mm/day; (++) 4-7 mm/day; (+++) 7 mm/day

<sup>c</sup>Siderophore activity on CAS medium: (+)1-5 mm wide of orange zone; (++) 5-20 mm wide of orange zone; (+++) 20 mm wide of orange zone.

Table 2. Appearance (%) of introduced bacterial strains in rhizosphere of different maize genotypes

Bacterial strain	Introduced strain patterns	Introduced strain pattern (%) in 5 maize genotypes				
		3014	4015	5043	6010	6030
<i>Bacillus</i> Q7	B-I	85	75	<b>95</b>	80	95
	B-A1	80	65	<b>95</b>	80	90
<i>Pseudomonas</i> PS2	P-II	65	70	<b>85</b>	65	65
	P-A2	55	45	<b>70</b>	50	55
<i>Azotobacter</i> NM5	A-III	<b>25</b>	80	85	70	75
	A-A1	<b>21</b>	5	5	5	10
<i>Azotobacter</i> NM8	A-III	25	80	<b>85</b>	70	75
	A-A2	2	75	<b>80</b>	65	65
<i>Azotobacter</i> NM14	A-IV	<b>75</b>	20	15	30	25
	A-A3	<b>75</b>	20	10	25	20

To develop the efficient mixture of strains for promoting maize growth and yield in field conditions we tested different maize genotypes and their impact on rhizosphere competence of applied strains. The higher percent of applied strain appearance was observed in *Bacillus* Q7 strain in combination with maize genotype 5043, than genotype 6030. Also, *Pseudomonas*



PS2 and *Azotobacter* NM8 were the most frequent in maize genotype 5043, while the maximal number of *Azotobacter* NM14 and *Azotobacter* NM5 was found in the rhizosphere of maize genotype 3014.

To achieve good growth promoting interaction between introduced PGP strains and maize genotype in the presence of other microorganisms, we estimated plant parameters: plant weight, 1000 seed weight, N, P and K content and yield (unpublished data).

The phosphate-solubilizing activity of microorganisms is enabled by production of organic and inorganic acids, converting insoluble mineral phosphates into soluble forms (Kravchenko et al., 2004). Solubilization of different form of phosphates often leads to increasing of mass and productivity of agriculture plants (Lugtenberg & Kamilova, 2009). *Pseudomonas* PS2 and *Azotobacter* NM8 showed substantial acid production and tricalcium phosphate solubilization. *Azotobacter* NM14 was able to solubilize this mineral phosphate very slowly (1.5 mm/day). Siderophore production was detected only by *Pseudomonas* PS2 strain and in several indigenous isolates. Isolate PS2 cause hyphal deformation of several phytopathogenic fungi (*Alternaria tenuissima*, *Curvularia lunata*, *Fusarium semitectum*, *F. equiseti* from *Salvia officinalis* L., *F. equiseti* from *Matricaria chamomilla* L., *Myrothecium verrucaria*, *Verticillium* sp., *Diaporthe eres complex* and *Sclerotinia sclerotiorum*) and effectively inhibit mycelial growth, due to production of chitinases, siderophores, lytic enzymes and several antibiotics (Uric et al., 2011; Jošić et al., 2012 a, b). All *Azotobacter* strains (Mrkova ki & Milic, 2001) and *Bacillus* Q7 strain (unpublished data) were able to stimulate growth of several agriculture plants. *Pseudomonas* PS2, being suitable for application in maize cultivation as biocontrol agent, and *Azotobacter* and *Bacillus* Q7, as good PGP strains, were involved in this competitiveness field trial investigation. All applied bacterial strains, except *Azotobacter* NM5, showed high percent of appearance in rhizosphere of all maize genotype and well adaptation to particular soil environment and extreme weather conditions (drought).

### Conclusion

The obtained results assessed by a combination of phenotyping and genotyping methods showed that a mixture of *Azotobacter* NM8 and NM14, *Pseudomonas* PS2 and *Bacillus* Q7 had better competitiveness to indigenous bacteria in the rhizosphere of all maize genotypes. The mixture can be further tested for application as maize growth and yield promoting inoculum on different soil types.

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**COMBINING ABILITY ANALYSIS OF ZP MAIZE INBRED LINES FOR GRAIN  
YIELD AND YIELD COMPONENTS**

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**Abstract**

A half diallel cross was used to evaluate combining abilities of six maize inbred lines and their hybrid combinations for grain yield, ear length, number of kernel rows per ear and number of kernels per row. Analysis was done using Griffing's formula (1956), method 2, mathematic model I. General and specific combining ability (GCA and SCA) mean squares were significant for all traits. GCA/SCA ratios revealed that additive gene effects had larger importance in inheritance of all of investigated traits than non-additive effects. Furthermore, obtained results showed that inbred lines L4 and L5 had the best GCA effects for grain yield, while inbred line L4 had the highest GCA values for ear length and number of kernels per row. Inbred line L6 was the best general combiner for number of kernel rows per ear. The hybrid combinations those exhibited significant SCA effects involved low x high, average x high and high x high GCA parents. According to obtained results, we could be concluded that inbred lines L4, L5 and L6 have high frequency of favorable alleles for most of the investigated traits and can be used in further breeding programs for new hybrids development.

**Key words:** maize, general and specific combining abilities, yield

**Introduction**

Maize is a one of the leading crops in worlds agricultural production. According to FAO statistical datas for 2011. year, 883.5 million tons of maize has been produced (<http://www.fao.org>). Serbia finds itself on 19. position on top twenty producing states in the world with 6.5 million produced in 2011. In order to follow world's increasing demands in production, creation of new and higher yielding hybrids is needed.

Such creation is achieved through evaluation of germplasm of unknown background. Whenever new and exotic germplasm is reached, it needs to be tested in order to see its breeding capacities and values. Such valuation is reached through field tests and statistical analysis of tested material. One of those techniques for testing material of uncertain genetic background is testing for combining abilities, introduced by Griffing (1956). Concept of combining abilities, even though, presented more than 50 years, still represents powerful tool for germplasm evaluation. It consists of general and specific combining abilities (GCA and SCA), where GCA stands for average value questioned trait of one parent crossed with other parents and SCA represents value of certain combination of two parents (Borojevic, 1981). GCA represents breeding value, ie. part of the variance that is fixed in parent components, so valuation through GCA represents selection of parents for creation of new populations and creation of new inbred lines. SCA, on the other hand, represents unreliable part of variance, as consequence of acting non-additive variance, which is happens in F1 offspring by crossing genetically distant inbred lines (Abuali et al. 2012). Observed genotypes with higher values of

combining abilities, can result in improved hybrids when they are crossed to other materials from the maize germplasm collection (amdžija et al. 2011).

### Materials and methods

Six selected inbred lines were chosen for testing the GCA/SCA performance. Based on empirical data it is assumed they belong to the same heterotic group. Lines are crossed in 2009<sup>th</sup> by the method of incomplete diallel without reciprocal combination [ $n(n-1)/2$ ]. As a result of derivative crossing, 15 hybrid combinations were obtained among the tested lines. The following year, inbred lines and hybrid combinations were tested in two separate experiments by RCB design at two locations in two replicates. Plot size was 6m<sup>2</sup>, and genotypes were sown at density of 69999 plants per hectare. Grain yield was measured for each elementary plot, and after harvesting and measuring the yield of each genotype, ten ears were selected from each replicate for analysis yield components. Yield components that were analysed were ear length, number of kernel rows and number of kernels per row. Analysis of combining ability was done by Griffing (1956), method 2, the mathematical model 1. All calculations were done in excel software.

### Results and discussion

It is determined by analysis of variance, in Table 1, that both GCA and SCA values were significantly high ( $p < 0,01$ ). GCA/SCA ratio was somewhat higher than unity for questioned traits. For grain yield and number of kernels per row ratio was little above unity (1), suggesting equal contribution of additive and non-additive genes, which is in accordance with Aguiar et al. (2003). On the other hand, highly significant values of GCA and SCA variances were found for ear length ( $p < 0,01$ ) and GCA/SCA ratio was 3.26 and 26.63 for ear length and number of kernel rows suggesting much greater role of additive genes in formation of these two traits in offspring. Similar results were found by Shalim et al. (2006) for ear length and Živanovic et al. (2010) for number of row kernels.

Table 1. Medium of squares of combining abilities of tested inbred lines

Source	df <sup>1</sup>	GY <sup>2</sup>	EL <sup>3</sup>	NKR <sup>4</sup>	NKPR <sup>5</sup>
GCA	5	5.90**	6.35**	8.50**	32.34**
SCA	15	5.13**	1.94**	0.32*	18.34**
Error	20	0.31	0.09	0.1	0.64
GCA/SCA		1.15	3.26	26.63	1.76

<sup>1</sup> degree of freedom; <sup>2</sup> grain yield; <sup>3</sup> ear length; <sup>4</sup> number of kernel rows; <sup>5</sup> number of kernels per row

Even though both variances were significant for number of kernels per row, additive genes were predominant, that conclusion is in accordance with Van etovic and Drinic (1993), who also found higher contribution of additive genes in heridation of the same trait.

Table 2. GCA values of tested inbred lines

Line	GY <sup>1</sup>	EL <sup>2</sup>	NKR <sup>3</sup>	NKPR <sup>4</sup>
L1	-0.69*	-0.29	-0.63**	-1.95**
L2	-0.78**	-0.63**	-0.38*	-2.01**
L3	-0.74*	-0.41*	-0.47**	-0.82
L4	1.05**	1.53**	-0.93**	3.23**
L5	0.93**	0.59**	0.58**	0.82
L6	0.24	-0.80**	1.83**	0.73
p<0.01	0.79	0.42	0.46	1.13
p<0.05	0.58	0.31	0.33	0.83

<sup>1</sup> grain yield; <sup>2</sup> ear length; <sup>3</sup> number of kernel rows; <sup>4</sup> number of kernels per row

Based on the data presented in Table 2 it can be seen that in inbred lines L4 and L5 recorded highly significant positive values of general combining ability for grain yield, indicating a high frequency of favorable alleles for grain yield in the two inbred lines. Inbred lines L4 and L5 had the highest values of GCA for ear length, while highly significant negative values were recorded in inbred lines L2 and L6. The highest value of GCA was observed in lines L6, and the lowest in lines L4, which was expected given that the value of the number of kernel rows per ear and ear length are negatively correlated (Zarei, 2012). Highly significant negative values of GCA for number of kernels per row had lines L1 and L2, and a highly significant positive value recorded in the line L4, which can therefore be used in further breeding programs as sources of genes for this certain trait.

Table 3. Values of specific combining ability of valued hybrid combinations

Genotype	GY <sup>1</sup>	EL <sup>2</sup>	NKR <sup>3</sup>	NKPR <sup>4</sup>
L1xL2	-0.43	-0.55	0.11	-0.58
L1xL3	-0.28	-0.31	-0.61	-0.53
L1xL4	2.09**	0.75*	0.06	3.27**
L1xL5	1.40	0.59*	0.14	1.74
L1xL6	1.80*	0.08	0.39	2.28**
L2xL3	0.13	-0.48	-0.16	-0.21
L2xL4	1.55*	0.99*	-0.50	3.57**
L2xL5	1.46*	0.83*	0.39	3.16**
L2xL6	0.44	0.42	-0.76	2.74*
L3xL4	0.86	0.57	0.09	-1.06
L3xL5	1.32	0.71	0.48	3.11**
L3xL6	1.03	0.50	0.03	2.50*
L4xL5	2.20**	2.27**	0.24	3.66**
L4xL6	0.97	1.76**	-0.91*	5.30**
L5xL6	1.70*	-0.35	0.38	0.47
p<0.01	1.93	1.03	1.11	2.78
p<0.05	1.41	0.75	0.82	2.03

<sup>1</sup> grain yield; <sup>2</sup> ear length; <sup>3</sup> number of kernel rows; <sup>4</sup> number of kernels per row

The highest SCA values for grain yield, as well as for ear length were recorded in hybrid combinations L4xL5 (Table 3), i.e. highest values were obtained by crossing the lines with the highest GCA values for the mentioned traits. The hybrid combination L3xL5 had highest SCA values for number of kernel rows, and it is a case of crossing of inbred lines with the negative and positive GCA value. Borojevic (1981) points out that the high SCA values are often obtained by crossing one parent with high GCA and one with low GCA. In hybrid combination L4xL6, the highest SCA value was recorded for the number of kernels per row. In this case, it is the combination of lines with a higher and average value of GCA.

### Conclusion

It can be concluded that inbred lines used in this research differ each other by examined traits. Depending on the goal of breeder, certain inbred line matches different breeding purpose. In this case, inbred lines L4 and L5 are recommended for breeding programme based directly on grain yield *per se*. L4 inbred line, also proved to be the best general combiner for ear length and number of kernels per row, so L4 line can be included in breeding programmes concerning those traits.

L6 was the best general combiner for number of kernel rows per ear. New and modern maize hybrids are characterized by greater number of kernel rows per ear, allowing greater number of kernels per ear. Concerning that fact, L6 inbred line should be involved in breeding programme concerning greater number of kernel rows per ear.

According to obtained results, we could be concluded that inbred lines L4, L5 and L6 have high frequency of favorable alleles for most of the investigated traits and should be used in further breeding programs for new hybrids development.

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**THE INFLUENCE OF MANY YEARS LIMING AND FERTILIZING TO  
CHANGING OF ADSORPTIVE COMPLEX COMPOSITION OF PSEUDOGLEY  
SOIL**

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**Abstract**

Composition and characteristics of soil adsorptive complex are very important to understand physicals and chemical processes which affect soil fertility and nutrients availability to plants. The paper deals with influence of many years ameliorative application of lime, manure and mineral fertilizers on adsorptive complex composition of pseudogley soil in Kraljevo valley. Soil belongs to group of extremely acid soil pseudogley type. Two – field crop rotation wheat – maize was applied. Soil samples were taken from opened profiles and numbered 1 (unfertilized variant – control), 2 (NPK) and 3 (NPK+CaCO<sub>3</sub>+manure). Arable lands, pseudogley type, have a high degree of dealcalinization of eluvial, -Ah and Eg-, layers and B<sub>1</sub>tg layer, too. Capacity value of cations exchange and saturation degree of exchangeable – adsorbed alkaline cations (Ca<sup>2+</sup>, Mg<sup>2+</sup> and H<sup>+</sup>(+Al) ions) varied considerable. Their composition and content, especially at -Ah and Eg layers, were unsatisfactory, mainly. According to noticed V – values (< 50%), analyzed soil belongs to group of "moderately unsaturated" soils. Many years, periodically, application of pedo–ameliorative treatments as: liming, phosphatization and humification, influenced increase of degree of alkali saturation (V%) and capacity of cations exchange (T) at Ah layer for more than 40% and 10 m.ekv./100 g soil, respectively. Part of alkaline cations (Ca<sup>2+</sup> and Mg<sup>2+</sup>) was increased, averagely, for 10 m.ekv./100 g soil or about 14.25% in relation with T values. Content of exchangeable – adsorbed K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> ions at eluvial horizons (Ah and Eg) was rather low, at analyzed soil profiles. Calcification caused increase of content of exchangeable Ca<sup>2+</sup>, Mg<sup>2+</sup> and K<sup>+</sup> in adsorptive complex of analyzed soil.

**Key words:** adsorptive complex, fertilization, liming, pseudogley, soil.

**Introduction**

Pseudogleys cover significant areas of Serbia, accounting for about 285,000 ha or 78.73% of the total land area in Western Serbia (Tanasijevic et al., 1966). These soils are found in moderately moist to moist climates, and they have disturbed water and air relationships characterised by an occasional decrease in very moist i.e. wet and dry phases. Therefore, this soil is unfavourable for the cultivation of most plants. The unfavourable soil moisture regime is due to the compact lower Btg horizon which is poorly permeable or impermeable. Under dry conditions, the soil surface horizon undergoes intense desiccation, whereas the deeper impermeable horizon hardens. During the wet phase, reduction conditions occur in the soil, resulting in the reduction of different elements, primarily iron (Fe<sup>3+</sup> to Fe<sup>2+</sup>), manganese, etc. Since the wet phase is short, only more susceptible substances undergo reduction. During the



dry phase, oxygen enters the soil, and oxidises the substances that were reduced during the wet phase ( $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$ ).

Pseudogleys are rather poor in alkalis, being medium to strongly acid in reaction. They have a highly unfavourable structure, and a low content of organic matter. The acid reaction of pseudogley, its low humus content, and a low supply with available phosphorus and potassium are limiting factors for higher crop yields (Dugalic et al., 2005).

Absence of carbonates at pseudogley has caused its intensive acidification that is confirmed by results of many authors for active, substitute and hydrolytic acidity from different regions (Kubiena, 1953; Muckenhausen, 1975; Pivic et al., 2011). Taking into consideration heterogeneity of pseudogley type, Škoric (1986) described dystic pseudogley form with very acid reaction at top part of profile (pH 5-5.5), little adsorption capacity and low alkali saturation (20-50%), for many regions of ex – Yugoslavia. At Bt horizon, acidity is slightly less, but adsorption capacity and alkali saturation are greater. There are, at significant concentrations, Al and Fe adsorbed ions, beside H ions. Humus is present in small amount, dominating fulvo acids in its composition (Dugalic, 1997).

The role of calcium in soil is well known. First of all, together with soil organic matter it influences on formation of stable structure and favorable conditions for microbiological processes in soil. In highly acid soils application of calcium maintain conditions for high efficiency of all agro-technical measures aimed for protection and increase of soil fertility. Application of Ca-containing materials and decrease of soil acidity reduce possibility (due to formation of insoluble forms) of higher uptake of toxic elements, which have tendencies of increased accumulation in soil due to anthropogenic pollution (Kabata-Pendias & Pendias, 2001). In addition to reducing acidity, application of lime, together with manure and conventional NPK fertilizers, affects increasing of sum of alkali cations at adsorptive soil complex (S), decreasing of potential acidity and increasing cation exchange capacity (CEC) as well as degree of saturation by exchangeable – adsorbed alkali cations (V%) (Pivic et al., 2011).

The aim of this investigation is to evaluate influence of many years application of some ameliorants to adsorptive complex composition at pseudogley in term of widely use of marked pedo – ameliorative treatments and fertilization for reducing acidity of that soil type in Serbia.

### **Materials and methods**

Investigation was conducted on stationary field fertilization trial, based at experimental field of the Dr. or e Radic Secondary School of Agriculture and Chemistry in Kraljevo 1995. Soil belongs to group of extremely acid soil pseudogley type. Two – field crop rotation wheat – maize was applied.

Experiment was set up by block system, in three replications, with area of elementary experimental plot  $100 \text{ m}^2$ . Amounts of nutrients, applied in trial, were:  $120 \text{ kg N ha}^{-1}$ ,  $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  and  $80 \text{ kg K}_2\text{O ha}^{-1}$ , used in form of NPK fertilizer (8:24:16), superphosphate (17%  $\text{P}_2\text{O}_5$ ) and ammonium-nitrate (AN=17% N). Lime fertilizer "Njival Ca", as product of Serbian glass factory from Paracin, was applied in experiment, too, at amount of  $4.0 \text{ t ha}^{-1}$ . Entire amounts of lime, phosphate and potassium fertilizers, together with 1/3 nitrogen one, were applied at per – sowing soil preparation, while the rest of nitrogen fertilizer was applied at spring beginning. Lime fertilizer was used periodically, each fifth year (1995, 2000, 2005 and 2010). Well burned out manure was used in amount of  $30 \text{ t ha}^{-1}$ . Other care procedures, implemented during vegetative period, were standard.

Soil samples were taken from opened profiles and numbered 1 (unfertilized variant – control), 2 (NPK) and 3 (NPK+CaCO<sub>3</sub>+manure). Samples were taken after wheat harvest 2012, from humus (Ah = 0-15 cm), underhumus, eluvial – pseudogley (Eg = 15-40 cm) and part of transitional B<sub>1</sub>tg horizons (60-80 cm).

Soil samples analyzes were done by standard chemical methods:

pH in water and KCl – electrometric method with glass electrode in suspension 1:2.5

humus content – Kodzmann method

sum adsorbed alkali cations – Kappena method

hidrolitic acidity – with Ca-acetate, Kappena method

degree of soil saturation by exchangeable – adsorbed cations – by calculating, by Hissink

exchangeable Ca and Mg ions and Na and K ions – by compleximetric and flamephotometric, respectively.

Results were presented by average values and statistically proceed by analyze of variance (Mead et al., 1996).

### Results and discussions

Agrochemical properties of pseudogley are very unfavorable (Table 1). Studied soil has high acidity. Active acidity at surface layer (0-20 cm) is relatively low (pH in H<sub>2</sub>O 5.24), while it is significantly decreased at the deepest soil layers (pH in H<sub>2</sub>O 6.04). Exchangeable acidity (pH in KCl) vary from 4.48 to 4.80, through soil depth. Value of hydrolytic acidity (Y<sup>1</sup>) at surface horizon (0-20 cm) is relatively high (15.47 ccm), while it is significantly decreased at deeper layers (7.35).

Table 1. Agrochemical properties of Pseudogley

Depth (cm)	Humus (%)	Y <sup>1</sup> (ccm)	N (%)	T	S	T-S	pH		P <sub>2</sub> O <sub>5</sub> (mg 100 <sup>-1</sup> )	K <sub>2</sub> O (mg 100 <sup>-1</sup> )
							H <sub>2</sub> O	KCl		
0-20	2.18	15.47	0.14	16.71	8.08	8.63	5.24	4.48	8.0	13.8
20-40	1.84	13.98	0.13	16.69	9.79	6.90	5.55	4.58	7.0	13.6
40-60	0.66	12.45	0.09	26.24	20.02	6.22	5.46	4.42	1.3	8.5
60-80	0.71	9.97	0.07	26.29	21.31	4.98	5.64	4.52	1.0	7.6
80-100	0.63	7.35	0.02	25.62	22.02	3.60	6.04	4.80	0.8	4.3

Studied soil type is characterized by very unfavorable composition of adsorptive complex (Table 1). Namely, values of adsorptive capacity, sum adsorbed alkali cations as well as degree of adsorptive complex saturation by alkalis are very low. Humus content at surface layer (0 – 20cm) is low, too, (2.18%) intending to decrease through soil depth. Such fact points out need to apply humification as necessary ameliorative treatment, as part of entire system of fertilization and applying pedo – ameliorative treatments.

Analyzed pseudogley profiles show medium nitrogen providing at humus – accumulative horizon. It is estimated that is average nitrogen content at surface layer 0.14%, decreasing significantly by soil depth increase (0.02%). This soil type is weakly provided by easy – available phosphorus (7.0-8.0 mg 100<sup>-1</sup> g, at 0 - 40 cm depht). On the contrary, content of easy – available potassium is satisfactory, putting this soil type in group of well – provided soil (13.8 mg 100 g<sup>-1</sup>).

Results for sum of alkali cations (S- values) point to high degree of dealkalization of studied profile part, with values below 21 m.e. 100 g<sup>-1</sup> (Table 2). Many years application of fertilizers

has led to minimal changes S – values at all horizons of studied soil. However, many years application of lime, manure and mineral fertilizers has caused noticeable increase of sum of exchangeable – adsorbed alkali cations (3.5-4.1 m.e. 100 g<sup>-1</sup> or 17.2-42.7%) in relate to control.

Capacity of cations exchange (T – values) varied significantly in pseudogley, in dependence to soil depth and fertilization variant (Table 2). T – values significantly increased going from Ah to B<sub>1</sub>tg profiles, due to the fact that it, at the low humus content, is the most dependent on clay content and composition. Namely, maximum capacity of cations exchange occurs at B<sub>1</sub>tg profile. Dugalic (1997) got similar results, investigating capacity of cations exchange at various pseudogley varieties in Kraljevo valley.

Table 2. Composition of adsorptive complex

Trea- Tment	S (m.e. 100 g <sup>-1</sup> )			T (m.e. 100 g <sup>-1</sup> )			T-S (m.e. 100 g <sup>-1</sup> )			V%		
	Horizons			Horizons			Horizons			Horizons		
	Ah	Eg	B <sub>1</sub> tg	Ah	Eg	B <sub>1</sub> tg	Ah	Eg	B <sub>1</sub> tg	Ah	Eg	B <sub>1</sub> tg
1.	9.6	20.3	20.9	19.4	29.6	33.1	9.8	9.3	12.2	47.7	50.5	62.8
2.	9.9	20.6	21.7	21.4	31.6	33.4	11.5	11.0	11.7	46.8	49.8	60.7
3.	13.7	23.8	24.7	27.9	38.4	34.6	14.2	14.6	9.9	58.0	58.6	60.0
Lsd 5%	0.31	1.03	0.16	0.24	0.94	1.76	1.41	1.05	1.01	3.42	0.05	0.91
1%	0.51	1.71	0.27	0.40	1.55	2.29	2.34	1.74	1.67	5.66	0.08	1.50

Many years application of fertilizers has caused increase of T – values at Ah and Eg horizons, while combined application of lime, manure and mineral fertilizers, through longer period, caused increase of T – values at Ah and Eg horizons for 43.8% and 29.7%., respectively, in relate to control. However, changes of T – values at B<sub>1</sub>tg horizon were minimal, under many years fertilization.

It can be seen (Table 2) that changes of T – values at all horizons of analyzed soil, are results of changes of capacity of cations exchange and sum of exchangeable – adsorbed alkali cations. Degree of saturation by exchangeable – adsorbed alkali cations (V%) is under 50% at humus – acumulative (Ah) horizon, on untreated land (control). Such date qualifies this soil type as "moderately unsaturated" soil. There is not significant difference between Eg and Ah horizons (50%) of analyzed soil profile in saturation by adsorbed alkali cations, on control plot. Significantly increase of V% value (62,8%) is noticed until at B<sub>1</sub>tg horizon (Table 2). So, it is, however, relatively low degree of saturation by adsorbed alkali cations, being, averagely, below 63%. Influence of many years, combined, application of lime, manure and mineral fertilizers on change degree of saturation by alkali cations is registered at Ah and Eg horizons of analyzed soil. These results are in accordance with results other authors about influence of combined fertilization on decrease soil acidity, increase adsorbed alkali cations and degree of saturation adsorptive complex by alkali cations (Dugalic, 1997; Pivic et al., 2011).

Content of exchangeable – adsorbed cations at profile of analyzed pseudogley varied significantly, as it can be seen in Table 3. So, content of exchangeable K<sup>+</sup> was increasing in line with increasing of soil depth. The lowest content (0.47 m.e. 100 g<sup>-1</sup>) was registered at Ah horizon, but the highest (0.58 m.e. 100 g<sup>-1</sup>) at B<sub>1</sub>tg one.

The part of exchangeable K<sup>+</sup> in adsorptive complex of Ah horizon has been increased for 31.9% in relate to control (1) as result of many years application lime, manure and NPK fertilizers (3). The most pronounced changes of exchangeable K<sup>+</sup> content have occurred at Ah and Eg pseudogley horizons.

Observing the depth profile, changes of part of exchangeable – adsorbed  $\text{Na}^+$  in soil, were relatively little, under the influence of many years fertilization. However, significantly varying of content of exchangeable – adsorbed  $\text{Ca}^{2+}$  ions at eluvial Ah and Eg pseudogley horizons were noticed.

Table 3. Exchangeable cations in the adsorptive complex (m.e.  $100 \text{ g}^{-1}$ )

Treat- ment	$\text{K}^+$			$\text{Na}^+$			$\text{Ca}^{2+}$			$\text{Mg}^{2+}$		
	Horizons			Horizons			Horizons			Horizons		
	Ah	Eg	B <sub>1</sub> tg	Ah	Eg	B <sub>1</sub> tg	Ah	Eg	B <sub>1</sub> tg	Ah	Eg	B <sub>1</sub> tg
1.	0.47	0.52	0.58	0.23	0.23	0.23	16.2	17.9	21.9	5.90	6.00	7.60
2.	0.59	0.59	0.62	0.21	0.21	0.28	16.4	18.2	22.3	5.40	7.20	9.80
3.	0.62	0.60	0.62	0.22	0.22	0.28	26.5	28.9	31.4	8.23	10.95	14.40
Lsd 5%	0.10	0.09	0.08	0.03	0.03	0.02	1.62	0.73	0.13	0.61	0.79	0.57
1%	0.17	0.15	0.13	0.06	0.06	0.03	2.68	1.22	0.22	1.02	1.30	0.94

Part of exchangeable  $\text{Ca}^{2+}$  ions in adsorptive complex of analyzed soil is rather low showing tendency to increase with increasing soil depth. The highest content of exchangeable  $\text{Ca}^{2+}$  at Ah and Eg horizons was result of many years, periodically application of lime, manure and NPK fertilizers. Achieved increase of exchangeable  $\text{Ca}^{2+}$  on mentioned fertilization variant (3) ranged over 60% in relate to control (1). The similar changes of content of exchangeable cations were obtained for  $\text{Mg}^{2+}$  ions, too (Tab. 3). It is evident calcification on extremely acid pseudogley cause increase of pH values of soil solution, leading to further generation of negative charge at surface of colloidal particles at adsorptive soil complex and, at the same time, increase of Ca, Mg and K cations adsorption (Ferreira Fontes and Ferracciú Alleoni, 2006).

### Conclusion

On the base of achieved results about composition of adsorptive complex of cultivated pseudogley variety and results about its improving by many years implementation of calcification, humification and fertilization, can be drawn following conclusions:

Analyzed pseudogley is very poor with exchangeable – adsorbed alkali cations, especially its humus – accumulative horizon (Ah), which is characterized by low average S - values ( $9.6 \text{ m.e. } 100 \text{ g}^{-1} \text{ soil}$ ) and T - values of capacity of cations exchange ( $19.4 \text{ m.e. } 100 \text{ g}^{-1} \text{ soil}$ ) increasing in line with soil depth. Many years combined application of lime, manure and NPK fertilizers resulted with increase S- values for 43.8% (Ah) and 29.7% (Eg) in relate to control and T- values from 17.2 to 42.7%.

Degree of soil saturation by exchangeable – adsorbed alkali cations (V%) was low, with average values 47.7% and 50.5% at Ah and Eg horizons, respectively. According to noticed V – values ( $< 50\%$ ), analyzed soil belongs to group of "moderately unsaturated" soils. Many years, combined application of lime, manure and mineral fertilizers affected significantly increase degree of soil saturation by alkali cations, especially at Ah and Eg horizons.

Content of exchangeable – adsorbed  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions at eluvial horizons (Ah and Eg) was rather low, at analyzed soil profiles. Calcification caused increase of content of exchangeable  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$  in adsorptive complex of analyzed soil.

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**CURRENT PROPAGATION OPTIONS FOR *MISCANTHUS GIGANTEUS* IN THE  
REPUBLIC OF SERBIA**

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**Abstract**

The key RS government's strategy for energy development is to expand the supply of home grown biomass and facilitate the development and competitiveness of a sustainable supply chain. Analysis of the potential supply chain suggests that this can partially be achieved by growing agroenergy crops. *Miscanthus giganteus* was chosen because of the potentially high productivity and cultivation on degraded soil.

This study aims to present the knowledge by which plant propagules (propagation) of *Miscanthus giganteus*, for the biomass supply chain, can be produced at minimum cost. Because *Miscanthus giganteus* is sterile, it can only be propagated by vegetative division.

The method of field experiments followed the potential of production of viable rhizomes on soils with variable fertility. Monitoring was done on 6 parameters of the rhizome growth and planting survival rate. The results indicate that the production of viable rhizomes is affected mainly by age of mother plants and biotic effects of the weed vegetation. A much smaller effect is shown through size of rhizomes and nursery fertilization.

The work reported here focuses on the available knowledge regarding the potential routes by which *Miscanthus* material could be mass produced for high density planting established to maximize yields. Vegetative clonal plant propagation is required to deliver uniform crops. Rhizome production and division is slow, but currently does not limit increase in production because the Serbian industry uptake is currently small.

At present the establishment rate of *Miscanthus* is slow and this appears to be limited by economics; evidence suggests that the cost of plant propagules is one factor that prevents widespread planting.

**Key words:** agroenergy crops, *Miscanthus giganteus*, ecoremediation

**Introduction**

*Miscanthus giganteus* is a highly productive plant species, which has been cultivated in Europe

for 20 years as energy crop. The remarkable adaptability of miscanthus to different environments

makes this novel crop suitable for establishment and distribution under a range of European and

North American climatic conditions (Lewandowski et al., 2000). It produces no seed, so it must be established vegetative by planting divided rhizome (rootstock) pieces. This process results in high establishment costs relative to crops established from seed.

There are two methods of propagation that are currently used for *Miscanthus* plants - rhizome division and micropropagation. The sterility of *M. x giganteus* necessitates vegetative propagation by either rhizome division or in vitro cultures. This results in very high costs for planting, making *Miscanthus* production economically non-viable. Furthermore, using a

single clone carries a considerable risk of attack from pests and diseases (Clifton- Brown et al., 2002).

Rhizome division is more used method because it is less expensive and generally produces more vigorous plants. To produce new planting material, two or three-year-old plants are split whilst dormant, using a rotary cultivator and the rhizome pieces collected for replanting.

A 30-40 fold increase in plants can be achieved this way over a period of 2-3 years, depending on soil conditions. Rhizome pieces must have at least 2-3 ‘buds’ and must be kept moist before re-planting. This is best achieved by keeping rhizomes under cold-storage conditions, (<4°C) (possibly for up to a year) but they will remain viable in the field for a short period of time, if stored in a heap and covered with moist soil.

This study aims to present the knowledge by which plant propagules of *Miscanthus giganteus*, for the biomass supply chain, can be produced in Republic of Serbia under agro-ecological conditions of fertile and degraded soils.

### **Material and methods**

Field experiments were established at following locations: 1. Sadzak (wetland); 2. Vrsac (fertile soil, chernozem - control field); and 3. RB Kolubara (landfil tailings). Plant material, rhizomes of *Miscanthus giganteus* (with a length of 10 cm and with 3 - 6 nodes), was purchased from commercial supplier and manually planted on agro-technically prepared land (plowing was conducted in the fall of the year before the planting and discing just before the planting). Investigated plates were:

A - without agrotechnical measures;

B - agrotechnical measures applied: watering during the 1st vegetation period, once just after planting and 4 times later; fertilization (N:P:K=15:15:15 150 kg/ha just before planting) and mechanical weed control 3 times per year.

At the locations Sadzak and Vrsac there has been a monitoring of the potential of rhizome production from 3, 2 and 1 year-old plants, (planting period in 2010, 2011 and 2012), and on humogley only 2 and 1 year-old (planting period in 2011 and 2012). Planting density was 5 rhizomes per m<sup>2</sup> and in every experiment there was a total of 40 plants and the presented results are arithmetic averages of 40 calculations. Measuring was done in the first part of April 2013.

A part, 50 rhizomes produced in this way were planted again at the same locations. Digging out, cutting and planting of rhizomes was done by hand. Rhizomes that have had sprouts within the 30 day period were considered to be viable.

In the second experiment, there was separate monitoring of the influence of irrigation, fertilization and weed on rhizome production from the 1<sup>st</sup> to the 3<sup>rd</sup> year on the experimental field Sadzak. Rhizomes that had 2, 4 or 6 sprouts were planted in April 2012. In the beginning of April 2013, rhizomes were dug out and cuttings, with 3-5 sprouts, were made for further planting. Other conditions were as in the previous experiment.

### **Results and discussion**

*Miscanthus* biomass yield can be limited by poor rhizome establishment and this is linked to rhizome age and storage conditions prior to planting. To avoid poor establishment, best practice recommends field planting directly after rhizome division. Operations avoiding rhizome storage, and utilizing favourable climatic conditions at planting, may be climatologically and logistically challenging when large areas are planted at high rhizome densities (Davies MJ, et al., 2011).

Our aim is to evaluate impacts of nursery age and soil type to produce and maintain rhizome viability when planted under conditions of fertile (Vrsac) and degraded soil (Sadzak and Kolubara).

Monitored locations are different primarily by soil characteristics (humogley in Sadzak, deposol in RB Kolubara, chernozem in Vrsac). The results from a rhizome establishment bioassay showed high viability.

Biometric characteristics shown in the Table 1 are for field experiment in which there were no agricultural measures applied, and Table 2. for the field experiment in which there was fertilization directly before planting, watering right after the planting and two more times during the first summer and mechanically removing weed three times during the first year of development and once during the second year.

At all three locations, the biggest production of rhizomes was from three years old nursery, and the lowest from the one from the last year, including agrotechnical measures. In the experiment without agrotechnical measures the biggest development of biomass above ground and underground was recorded in Vrsac which was expected considering soil fertility. Namely, at that location, measured content of humus was 1.71% which is significantly more than at other locations (Sadzak 0.60% and Kolubara 0.64%). With the application of agrotechnical measures parameters of *Miscanthus* development at the experimental field Sadzak are getting close to the values of the experimental field in Vrsac, especially for rhizomes produced from three year plants (Table 2). The results of the experiment in Kolubara show that the *Miscanthus* development is significantly limited without agrotechnical measures even though the canopy survives in these extreme conditions. With irrigation, fertilization and weed removing, results are becoming comparable with the ones acquired in other locations. All three locations show that rhizomes are mostly viable from over 50% of rhizomes from the nursery at Kolubara to almost 95% for rhizomes produced from three year nursery at Sadzak with measures.

Table 1. *Without agrotechnical measures*

	SADZAK			VRSAC			KOLUBARA	
	III	II	I	III	II	I	II	I
Tillering (nu)	54	36	16	58	38	18	24	8
Clump diameter (cm)	46	40	28	52	34	26	36	24
Whole length of rhizome	330	160	110	460	240	90	80	50
Number of rhizomecutings	52	40	24	68	56	32	30	14
Rhizomes survived (%)	88	85	72	90	88	76	66	52

Table 2. *With agricultural measures*

	SADZAK			VRSAC			KOLUBARA	
	III	II	I	III	II	I	II	I
Tillering (nu)	70	52	20	60	38	32	48	24
Clump diameter (cm)	60	54	36	66	50	42	46	30
Whole length of rhizome	580	340	220	600	350	180	280	160
Number of rhizome cutings	75	56	32	68	52	30	42	28
rhizomes survived (%)	94	88	80	92	90	86	82	70

Acquired results show that on degraded, swamp soil, as the Sadzak location, yields of viable rhizomes can be almost as the ones on fertile soil (Vrsac). Similar results were also achieved for biomass yield (Dražić, G. et al., 2012) and the possible reason is that the conditions on this site are similar to the natural conditions in which *Miscanthus* grows (Ji-Hoon Chung et al.,



2012), and the raise in temperatures is good for this agroenergetic crop. In a different experiment, monitoring was done on the influence of rhizome size, irrigation, fertilization and weed control on a production of new rhizomes. The production of rhizomes was mostly influenced by the competition with weed not considering the size of primer cutting (Table 3). On the other hand, Primer cuttings that had only two buds produced clumbs with significantly smaller rhizomes that the ones with 4 and 6 buds. Survival of the rhizomes planted in 2012 also depends on the age of nursery and on conditions of development from which the weed vegetation is considered to be the most important negative factor. Similar results were achieved for development of biomass above ground (Dražić, G. et al., 2010).

Table 3. The number of survived rhizomes

	1 <sup>st</sup> year			3 <sup>rd</sup> year		
	**2 buds	4 buds	6 buds	2 buds	4 buds	6 buds
*Control	12	24	25	20	52	56
Watering	18	25	25	36	61	68
Fertilizing	14	26	28	40	60	66
Weed control	20	33	35	58	72	80

\* without any agricultural measures

\*\* number of buds on rhizomes at planting

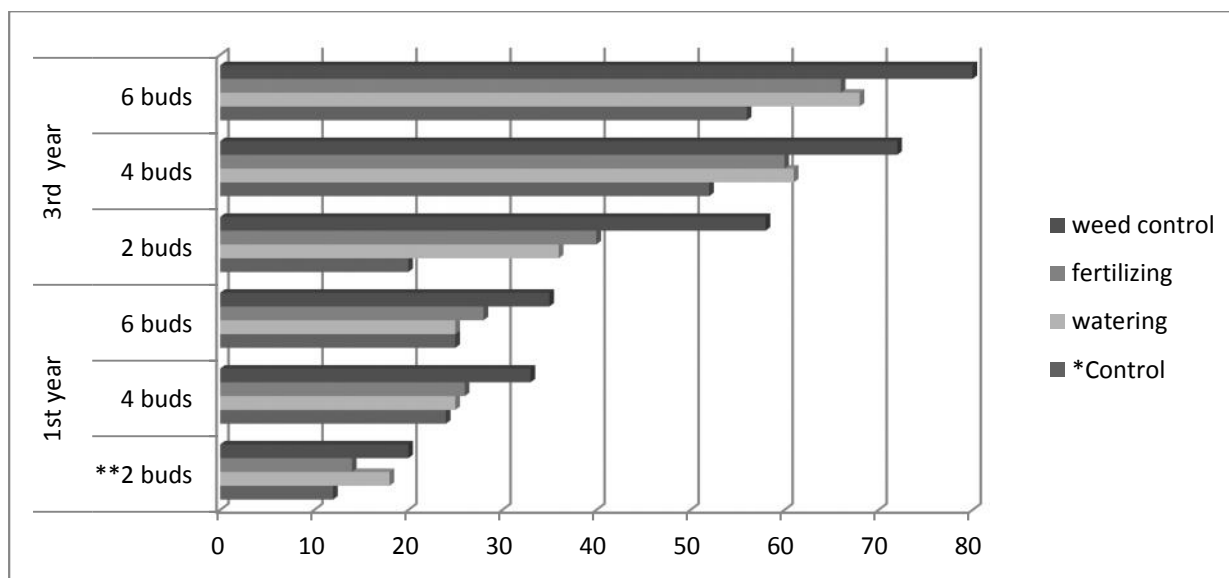


Chart 1. The number of rhizome cuttings

Table 4. Survived rhizomes (2012/2013) %

	1 <sup>st</sup> year			3 <sup>rd</sup> year		
	**2 buds	4 buds	6 buds	2 buds	4 buds	6 buds
*Control	32	54	55	64	85	84
Watering	38	55	56	56	90	92
Fertilizing	42	26	78	50	90	92
Weed control	44	78	82	68	94	98

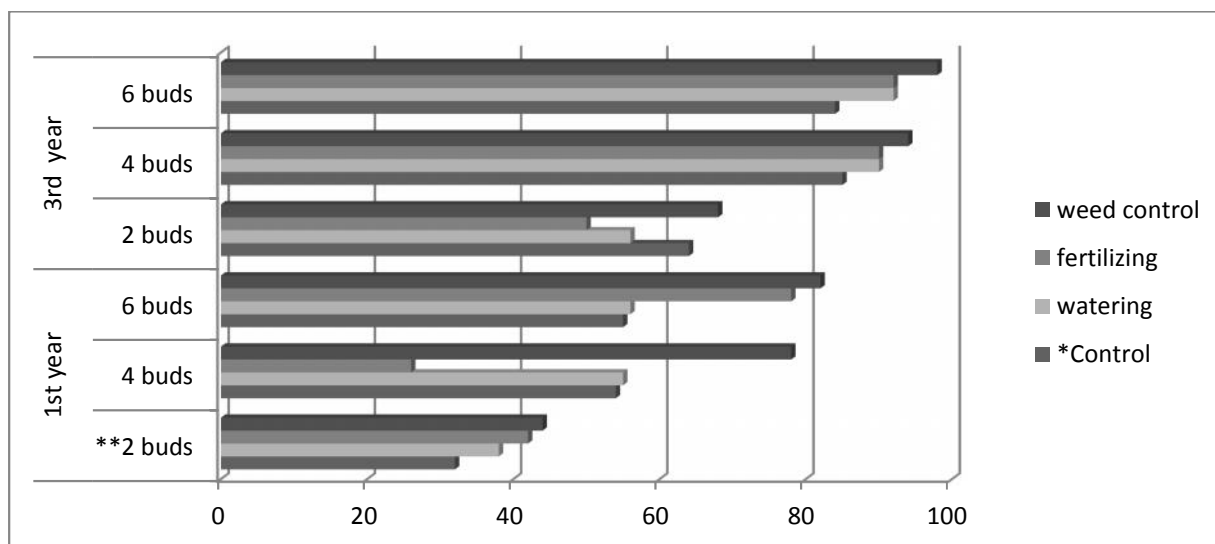


Chart 2. *Survived rhizomes (2012/2013) in %*

At all three locations the competition with weed significantly decreases rhizome yield. Similar results are achieved for biomass above ground (Milovanovic, J. et al., 2012). A large amount of weed (especially at Sadzak and Kolubara sites) is a consequence of the lack of weed control with total pesticide in the year before planting: in Sadzak, before *Miscanthus*, the soil was used for agricultural purposes with corn and in Kolubara it was a landfill and razastrto before planting. The results achieved at the experimental field Kolubara, even though they refer to only two vegetation show that, even in the bad conditions like these, *Miscanthus* development is possible, which opens possibilities for further researches for increasing production of underground as well as aboveground parts of the plant.

During the establishing of *Miscanthus* plantation as a base for bioenergy production chain, the biggest cost is starting plantaza, i.e. acquiring of planting material (Dražić, G. et al., 2010).

The results presented here point out to a possibility of establishing a nursery on relatively small surfaces, from which viable rhizomes could be produced. With a simple calculation (for example for the Sadzak site from a three-year-old nursery from one till that developed from one rhizome, one can get 75 rhizomes, and as 5 rhizomes are planted on a  $1\text{m}^2$  and the survival rate is 94% one can get 350 viable rhizome cuttings per  $\text{m}^2$ . If the density of planting *Miscanthus* is meant for biomass production, 1 rhizome/ $\text{m}^2$ , i.e. 10 000 rhizomes/ha, it means that less than  $30\text{m}^2$  of nursery is enough for 1 ha of field. The price of establishing *Miscanthus* plantation is around 3000 €/ha of which the largest part is a price of the rhizomes of 0,18 €/unit. With this price, the field that lasts 20 years with the average biomass yield of 20 t/ha and production and distribution of biomass briketi for 130 €/t economic analysis show the Return on Capital Employed –ROCE in EUR =447,07 %; Working capital turnover - WCT = 9,82. If there was a nursery production these indicators would be much better.

The results of this research are completely the same as the latest publications: “A review of current propagation options for *Miscanthus*”[9]. At present the establishment rate of *Miscanthus* is slow and this appears limited by economics; evidence suggests that the cost of plant propagules is one factor that constrains widespread planting. New techniques are required that simultaneously reduce unit costs of propagules and increase the speed of their availability to aid this developing industry.

### Conclusion

In agroecological conditions of the Republic of Serbia it is possible to produce viable rhizomes of *Miscanthus giganteus*

Production rate depends on soil fertility and application of agrotechnical measures

It is possible to achieve results on degraded soil close to the ones on fertile soil

The production of planting material of *Miscanthus* in private nursery significantly reduces the cost of establishing a plantation and in that way the whole production chain becomes economically acceptable

### Acknowledgement

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## **WATER USE EFFICIENCY OF TOMATO AND POTATO IN THE CONDITIONS OF SOUTHERN SERBIA**

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### **Abstract**

Water is a limited agricultural resource, so this study has been related to rational use of water in the intensive tomato and potato growing technology. By setting irrigation at different values of SWP (soil water potential), it have been studied their effects on yield, evapotranspiration and water use efficiency (WUE) of tomato and potato in the conditions of southern Serbia. The four-year investigation was carried out by a biological procedure – through field trials in the conditions with irrigation of tomato hybrid Amati F<sub>1</sub> and potato cultivar Kennebec, on alluvium soil type, in the river valley of Southern Morava, near Niš. Local coordinates of the studied area were the following: latitude 43° 19', longitude 21° 54', and altitude 194 m. The experimental field consisted of three treatments with irrigation (SWP of 20, 30 and 40 kPa), as well as unirrigated control. Tensiometers were installed at the depth of 20 cm within root system zone, and were read twice a day at 8<sup>00</sup> and 18<sup>00</sup>. Irrigation was applied when a lower value than predetermined was read on the vacuummeter. The highest value of WUE in tomato (112.68 kg ha<sup>-1</sup> mm<sup>-1</sup>) was observed in 2006 at the variant with SWP of 30 kPa, while the lowest one (77.27 kg ha<sup>-1</sup> mm<sup>-1</sup>) was observed in 2007 at the variant with SWP of 40 kPa. Water use efficiency of potato during the studied period ranged from 81.23 to 98.21 kg ha<sup>-1</sup> mm<sup>-1</sup>.

**Key words:** soil water potential, tomato, potato, irrigation, water use efficiency.

### **Introduction**

Climatic conditions of southern Serbia in the last few years were characterized by long drought periods without precipitation, and extremely high temperature during vegetation period. Optimal soil moisture for growing agricultural crops can only be reached in the conditions of irrigation. Insight in values of potential evapotranspiration (PET), or water demands by plants, is a necessary precondition for realizing an efficient water regime. Excessive irrigation leads to deep percolation of nutrients, higher potential for appearing plant diseases and pests, deterioration of soil structure and water losses, causing increased production costs. Determining irrigation term is important, because it is necessary to ensure the optimal soil moisture during vegetation, in order to supply the plants with enough available water. There are many methods of deciding irrigation term, but in agricultural practice the methods based on soil moisture measuring are regarded as the most reliable ones. The most frequently used device for soil moisture measuring in irrigation practice is tensiometer. Efficiency of this method for deciding terms of vegetable crops irrigation is confirmed by numerous reports (Clark et al., 1994; Smajstrla and Locascio, 1996; Li et al., 1998; Shock et al., 2000; Wang et al., 2004; Kang et al., 2004; Kang and Wan, 2005; Muñoz-Carpena et al., 2004; etc.). Water is a limited agricultural resource, so this study has been

related to rational use of water in the intensive tomato and potato growing technology. By setting irrigation at different values of SWP (soil water potential), it have been studied their effects on fruit and tuber yield, evapotranspiration, water use efficiency (WUE), and irrigation water use efficiency (IWUE) of tomato and potato in the conditions of southern Serbia.

### Material and methods

The four-year investigation was carried out by a biological procedure – through field trials in the conditions with irrigation of tomato hybrid Amati F<sub>1</sub> and potato cultivar Kennebec, on alluvium soil type, in the river valley of Southern Morava, near Niš. Local coordinates of the studied area were the following: latitude 43° 19', longitude 21° 54', and altitude 194 m. The experiments were set in random complete block design with four replications, where three irrigation variants were involved (SWP of 20 kPa, 30 kPa, 40 kPa) together with the unirrigated control. Irrigation was carried out by the drip irrigation system. Tensiometers were installed at the depth of 20 cm within root system zone, and were read twice a day at 8<sup>00</sup> and 18<sup>00</sup>. Irrigation was applied when a lower value than predetermined was read on the vacuummeter.

Tomato was planted within optimal agrotechnical terms and contemporary tomato growing technology was applied. Elementary plot area was 10.5 m<sup>2</sup>, with inter-row distance of 70 cm and within-row distance of 30 cm. After doing soil chemical analyses, basic amount of fertilizers was applied, and additional fertilization during vegetation period was done by fertigation. The total amounts of the applied nutrients were as follows: N – 283 kg ha<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub> – 187 kg ha<sup>-1</sup>, K<sub>2</sub>O – 525 kg ha<sup>-1</sup>, and MgO – 95 kg ha<sup>-1</sup>.

Potato planting (distance 70 cm between rows and 30 cm in the row) was done in the first half of April in both investigation years, with the cultivar Kennebec, original category, where tuber size was from 35-55 mm. After soil chemical analyses soil was fertilized before cultivation, as well as during vegetation by water soluble fertilizers through irrigation systems. The total amount of nutrients deposited to soil was: N – 200 kg ha<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub> – 120 kg ha<sup>-1</sup>, K<sub>2</sub>O – 300 kg ha<sup>-1</sup>, CaO – 100 kg ha<sup>-1</sup>, and MgO – 60 kg ha<sup>-1</sup>. During vegetation, the all modern agrotechnique measures were applied, and tuber harvest was carried out in the third decade of August in both years of the study.

Calculation of water consumption for evapotranspiration in the conditions of irrigation was done for each month and for vegetation period in whole (1), by balancing water from precipitation during vegetation period, soil supplies (2), irrigation, and potentially percolated or flown out water after heavy rains (3). Precipitation was measured by a rain gauge at the experimental field.

$$ET_{vp} = (W_1 - W_2) + P + I - D \text{ (mm)} \quad (1)$$

where  $ET_{vp}$  is evapotranspiration for the vegetation period;  $W_1$  is amount of water in soil to the depth of 1.2 m at the beginning of vegetation;  $W_2$  is amount of water in soil to the depth of 1.2 m at the end of vegetation;  $P$  is water amount from precipitation;  $I$  is water amount from irrigation;  $D$  is water loss by deep percolation and runoff.

$$W = 100 \cdot h \cdot d \cdot s \text{ (mm)} \quad (2)$$

where  $W$  is amount of water in soil to the depth of 1.2 m;  $h$  is depth of soil;  $d$  is bulk density;  $s$  is soil moisture.

Following heavy precipitation, water percolation into deeper soil layers and runoff was calculated:

$$D = (W_1 + P) - FWC \text{ (mm)} \quad (3)$$

where  $D$  is deep percolation;  $W_1$  is soil water amount to the depth of 1.2 m at the beginning of vegetation;  $P$  is precipitation amount (mm);  $FWC$  is field water capacity.

Rationality of water consumption is measured by water use efficiency (WUE) of tomato and potato. WUE is relationship between water consumption for evapotranspiration (ET) and yield, calculated as tomato fruit yield or potato tuber yield divided by ET. Irrigated water use efficiency (IWUE) was calculated as irrigated yield minus non-irrigated yield (control) divided by irrigation water amount (Schneider and Howell, 1998).

Data of tomato fruit yield and potato tuber yield were processed by analysis of variance, and significance of differences in yield was determined by comparing them with LSD values for  $P < 0.05$  and  $P < 0.01$ .

#### Mechanical and water-physical properties of soil in the experimental field

The obtained values of texture analysis (table 1) were expected, because fractional relations confirm that this is a loamy alluvial soil.

Table 1. Mechanical properties of soil

Depth (cm)	Total sand (%)	Powder (%)	Clay (%)
	> 0.02 mm	0.02-0.002 mm	< 0.002 mm
0-20	42.1	40.5	17.4
20-40	40.3	37.8	21.9
40-60	38.7	36.3	25.0
60-80	36.7	35.9	27.4
80-100	35.1	32.3	32.6
100-120	33.6	29.7	36.7

Immediately before the study began, water-physical properties of soil in the experimental field were determined (table 2).

Table 2. Water-physical properties of soil

Depth (cm)	FWC (weight %)	Specific weight ( $\text{g cm}^{-3}$ )	Bulk density ( $\text{g cm}^{-3}$ )	Total porosity (vol.%)	Capacity for water (vol. %)	Capacity for air (vol. %)
0-20	27.32	2.65	1.35	49.05	36.88	12.17
20-40	25.94	2.58	1.34	48.06	34.76	13.30
40-60	24.44	2.56	1.34	47.65	32.75	14.90

### Results and discussion

The average water consumption of tomato for evapotranspiration, observing the whole investigated period, was 621.2 at the variant with SWP of 20 kPa, 583.9 mm at the variant with SWP of 30 kPa, and 556.5 at the variant with SWP of 40 kPa (table 3). During the vegetation period of 2007 higher ET values were measured in regard to 2006, at the all irrigated variants, which could be explained by a higher average temperature. Although higher ET values were observed in 2007 than in 2006 in the conditions of irrigation, it did not affect tomato fruit yield. The measured tomato evapotranspiration in the conditions of irrigation was between 555.9 and 637.6 mm. The greatest tomato fruit yield was observed when the average water consumption for ETP amounted 584 mm, so this value could be regarded as tomato's demand for water in southern Serbia.

Similar values of tomato ET were reported by Pruitt et al. (1984), who by lysimeters measured water consumption of 515.8-614.7 mm for ET. Our values of tomato ET are greater than ET values (450-520 mm) found by Bošnjak and Pejic (1995) for the conditions of Vojvodina (Serbia). Hanson and May (2006), by the four-year investigation in the conditions of California, determined tomato water consumption for evapotranspiration of 528-752 mm, and the average value of ETP was 648 mm. Significantly lower tomato water consumption for ET (200-270 mm) in regard to our study was reported by Wang et al., (2007) for the conditions of North China Plain.

The two-year study showed high-significantly greater tomato fruit yield in the conditions of irrigation in regard to the unirrigated control (table 3). At the irrigation variant with SWP of 30 kPa also was reached high-significantly greater tomato fruit yield in regard to the variants with SWP of 20 and 40 kPa. Between the irrigation variants with SWP of 20 and 40 kPa there was not any significant difference. Highly significant difference in tomato fruit yield between the investigated years was observed at the all studied variants.

Table 3. Evapotranspiration, fruit yield, WUE, and IWUE of tomato

Year	SWP (kPa)	Soil water supplies (mm)	P (mm)	I (mm)	ET (mm)	Fruit yield (kg ha <sup>-1</sup> )	WUE (kg ha <sup>-1</sup> mm <sup>-1</sup> )	IWUE (kg ha <sup>-1</sup> mm <sup>-1</sup> )
2006	20	31.4	229.4	400	604.8	58146	96.14	53.63
	30	44.0	229.4	340	573.4	64614	112.68	82.12
	40	56.5	229.4	320	555.9	60627	109.06	74.79
	Control	78.3	229.4	-	307.7	36693	119.25	-
2007	20	33.4	208.2	440	637.6	46532	78.28	53.05
	30	40.2	208.2	390	594.4	53268	89.16	77.13
	40	56.9	208.2	340	557.1	43051	77.27	58.42
	Control	83.1	208.2	-	291.3	23189	79.60	-
LSD (yield)			SWP		Year		SWP x Year	
0.05			2813.5		1989.5		3978.8	
0.01			3796.9		2684.9		5369.6	

Water use efficiency (WUE) of tomato was much higher in 2006 in regard to 2007 (table 3). The highest value of WUE (112.68 kg ha<sup>-1</sup> mm<sup>-1</sup>) was observed in 2006 at the variant with SWP of 30 kPa, while the lowest one (77.27 kg ha<sup>-1</sup> mm<sup>-1</sup>) was observed in 2007 at the variant with SWP of 40 kPa.

Calculated average value of IWUE (70.18 kg ha<sup>-1</sup> mm<sup>-1</sup>) in 2006 was higher than the average value of IWUE (62.87) in 2007. The lowest value of IWUE was noted in 2007 at the variant with SWP of 20 kPa, while the highest IWUE value was detected in 2006 at the variant with SWP of 30 kPa.

The established value of evapotranspiration of potato in our experimental field ranged from 294.4 mm at the variant without irrigation to 522.1 mm at the irrigated variant with SWP of 20 kPa (table 4). According to many researches water demands of potato vary over a great range, depending above all on studied environment. For high tuber yield vegetation demands of potato for water ranged from 500 to 700 mm, depending on climatic conditions (Doorenbos and Kassam, 1979). During a three-year investigation potato evapotranspiration in Wisconsin (USA) was between 293 and 405 mm (Tanner, 1981). Wright and Stark (1990) stated water consumption of potato for evapotranspiration from 640 to 700 mm in irrigated areas of

Oregon and Washington (USA). Pereira et al. (1995) found potato evapotranspiration of 283 mm. Kiziloglu et al. (2006) stated that in the conditions of Erzurum (Turkey) potato evapotranspiration ranged from 167 mm without irrigation to 610 mm in the conditions of irrigation. According to Erdem et al. (2006) in Trakia Region (Turkey) potato evapotranspiration was between 464 and 683 mm.

The highest and also stable tuber yield in the two-year period of study was reached when water consumption for evapotranspiration was between 491.3 and 498.6 mm, so that value could be considered as potential evapotranspiration (PET) of potato, i.e. its water demands in the conditions of southern Serbia. The measured value of potato PET in our study is greater in regard to the value of potential evapotranspiration (460-480 mm) established by Bošnjak and Pejic (1994) for the conditions of Vojvodina Province (northern Serbia).

Concerning the all irrigated variants, tuber yield was high-significantly higher in regard to the unirrigated control. The highest potato tuber yield was observed at the variant with soil water potential of 30 kPa (table 4). Statistically high-significantly important differences in tuber yield were observed between the treatment with SWP of 30 kPa and the treatments with SWP of 20 and 40 kPa. However, at the treatment with SWP of 20 kPa, tuber yield was significantly higher regarding the treatment with SWP of 40 kPa.

Table 4. Evapotranspiration, tuber yield, WUE and IWUE of potato

Year	SWP (kPa)	Soil water supplies (mm)	P (mm)	I (mm)	ET (mm)	Tuber yield (kg ha <sup>-1</sup> )	WUE (kg ha <sup>-1</sup> mm <sup>-1</sup> )	IWUE (kg ha <sup>-1</sup> mm <sup>-1</sup> )
2008	20	21.4	222.7	278	522.1	44710	85.63	70.68
	30	32.6	222.7	236	491.3	47640	96.97	97.96
	40	51.5	222.7	175	449.2	36490	81.23	68.40
	Control	65.4	222.7	-	288.1	24520	85.11	-
2009	20	27.8	231.2	256	515.0	45480	88.31	73.05
	30	48.4	231.2	219	498,6	48970	98.21	101.32
	40	53.1	231.2	184	468,3	39130	83.56	67.12
	Control	63.2	231.2	-	294.4	26780	90.96	-
LSD (yield)			SWP		Year		SWP x Year	
0.05			1950		1390		2760	
0.01			2150		1540		3050	

In the conditions of irrigation the highest mean value of potato WUE of 97.59 kg ha<sup>-1</sup> mm<sup>-1</sup> was observed at the variant with SWP of 30 kPa (table 4). WUE values of potato obtained by this study (from 81.23 to 98.21 kg ha<sup>-1</sup> mm<sup>-1</sup>) were similar to the values reported by Wright and Stark (1990) and Beheral and Panda (2009).

Our values of potato WUE are not in accordance with the values stated by Wang et al. (2006) from 50.4 to 77.1 kg ha<sup>-1</sup> mm<sup>-1</sup> in season 2001 and 103.2-131.6 kg h<sup>-1</sup>a mm<sup>-1</sup> in season 2002 in the conditions of North China Plain. Values of WUE in our study were higher than the ones determined by Kiziloglu et al. (2006), which were from 40.2 to 63.4 kg ha<sup>-1</sup> mm<sup>-1</sup>, as well as the ones of Rashidi and Gholami (2008) who stated WUE ranging from 19.2 to 52.5 kg ha<sup>-1</sup> mm<sup>-1</sup>.



### Conclusion

The highest values of WUE and IWUE for tomato and potato were reached in the variant with SWP of 30 kPa, meaning rational water consumption was enabled at this SWP value. Evapotranspiration values vary widely, which above all depends on climatic conditions, soil texture and area of investigation. Therefore, the established value of potential evapotranspiration of tomato and potato is important for irrigation practice in southern Serbia or in areas of similar soil and climatic conditions.

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## THE POSSIBILITIES OF USE OF NITROGEN HARVEST INDEX IN WHEAT BREEDING IN TERM OF ECOLOGICAL AGRICULTURE

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### Abstract

The topic of nitrogen wheat nutrition was becoming very actually during last decades of last century because of many reasons: fertilizers price, energetic crises, environmental protection, ecological agriculture. Despite the detrimental impacts, the use of fertilizers (N in particular) in agriculture, together with an improvement in cropping systems, mainly in developed countries, have provided a food supply sufficient for both animal and human consumption. Therefore, the challenge for the next decades, with an expanding world population, will be to develop a highly productive agriculture, whilst at the same time preserving the quality of the environment. A multidisciplinary approach to breeding winter wheat and include physiological indicators of nitrogen nutrition efficiency could help in achieving this goal. Consequently, this paper deals with physiological indicator as nitrogen harvest index, its connection with grain yield, heritability and variance and evaluation of Serbian winter wheat genotypes in term of this indicator. The best values of nitrogen harvest index were registered at KG 165/2, Pobeda and Bujna. Emphasized genotypes, selected as superior in term of this indicator, could be considered as carriers of desirable traits in terms of wheat breeding theory, improvement of production efficiency, environmental protection and development of ecological agriculture.

**Key words:** breeding, ecological agriculture, nitrogen harvest index, wheat.

### Introduction

Despite the detrimental impacts, including pollution of soil and water and deterioration of food safety, the use of fertilizers (N in particular) in agriculture, together with an improvement in cropping systems, mainly in developed countries, have provided a food supply sufficient for both animal and human consumption. Therefore, the challenge for the next decades, with an expanding world population, will be to develop a highly productive agriculture, whilst at the same time preserving the quality of the environment (Hirel et al., 2001; Patel et al., 2004; Shrawat et al., 2008; Weinkauf, 2008). One way to enhance productivity and maintain efficient production and minimize environmental impact is to develop specific cropping strategies and select productive genotypes that can grow under low N conditions (Delmer, 2005). A multidisciplinary approach to breeding winter wheat and include physiological indicators of nitrogen nutrition efficiency could help in achieving this goal (van Ginke et al., 2001; Baker et al., 2004; Flowers et al., 2004; Zivanovic et al., 2006; Pathak et al., 2008).

The concept of the nitrogen nutrition efficiency of a crop should be considered a function of root activity, soil texture, climate conditions, interactions between soil and bacterial processes, the nature of organic or inorganic N sources and genetics specificity (Walley et al., 2002; Burger & Jackson 2004; Lopez - Bellido et al., 2005; Haberle et al., 2006). The direct evaluation of root system activity as a measure of plant absorption efficiency is difficult under

field conditions indirect measurements are possible. The relationship between indicators of absorption efficiency as well as nitrogen utilization ones and desirable traits such as grain yield in this case is an important question. Many authors (Anderson et al., 2004; Gallais & Coque, 2005) have defined parameters that affect grain yield positively.

Nitrogen harvest index (the ratio of nitrogen content in grain and in the whole plant) is a measure of the efficiency of nitrogen translocation from vegetative organs to the grains. Nitrogen harvest index reflects the grain protein content and thus the grain nutrition quality (Hirel et al., 2007) and, for wheat, usually ranges from 0.70 to 0.80 (Brancourt - Hummel et al., 2003).

The objective of this study was to investigate phenotypic variability of nitrogen harvest index and its relationship with grain yield in thirty bread wheat genotypes, grown under different environmental conditions during three year, at sub – optimal nitrogen soil provided.

### Material and method

The study was carried out on the property of the Small Grains Research Center in Kragujevac city (186 m.a.s.l.) in Serbia, during the three consecutive seasons (2001/02, 2002/03 and 2003/04). The soil type was smonitza in degradation (Vertisol).

The average temperatures and monthly rainfall during the wheat vegetation period (October-June) for the three seasons and the 30 years mean (1970-2000) are shown in table 1. In all three years, the mean temperature was higher than the 30 yr average. There was considerable variability in rainfall amounts and distribution from year to year. The amount of rainfall was most suitable for plant growth in the third season. Rainfall (74.5mm), received during the germination period (October - November) in the first season was less than in other two (97.00mm and 111.8mm) and long-term average (94.73 mm). Rainfall distribution during the rest of the vegetative period in the first season was auspicious but the total amounts of rainfall were less than long – term means.

Table 1. Weather conditions during the three test growing season and long-term (30-yr) mean for (LTM) winter wheat

Month	Average monthly temperatures ( C)				Monthly amounts of rainfall (l)			
	2001/02	2002/03	2003/04	LTM	2001/02	2002/03	2003/04	LTM
X	13.8	12.2	10.6	11.40	10.4	65.5	83.2	47.53
XI	4.6	9.7	8.9	5.90	64.1	31.5	28.6	47.20
XII	- 2.4	1.1	2.2	2.13	27.6	39.4	37.2	44.33
I	- 0.1	0.7	- 0.9	0.73	17.2	59.0	86.4	36.70
II	7.0	- 2.4	3.0	2.42	20.1	19.7	59.5	35.77
III	8.9	5.8	7.1	6.43	26.0	2.8	21.3	41.57
IV	10.8	10.8	12.8	11.22	63.7	37.2	52.3	50.77
V	18.4	19.9	14.5	16.24	38.6	42.3	50.3	65.43
VI	21.6	23.3	19.8	19.40	57.2	47.7	61.4	81.27
Season average					Total			
	9.18	9.01	8.67	8.43	324.9	345.1	483.2	624.43

The experiment included 30 wheat cultivars and experimental lines, originating from the Serbia: Small Grains Research Center, Kragujevac and Institute of Field and Vegetable Crops, Novi Sad. The basic processing and pre – sowing preparation of the soil was done using

standard procedures. The randomized complete block experimental design was used with five replicates in rows 1.5m on, with spacing between rows of 0.20m. Sowing (200 grains per row) was done by hand (one genotype per row), during the optimal planting period for central Serbian conditions, for winter wheat (29. 10. 2001, 15. 11. 2002 and 06.11. 2003). NPK fertilizer, formulated 8:24:16, was applied at the rate of 300 kg ha<sup>-1</sup> before sowing each season. Eight grams row<sup>-1</sup> of nitrogen (260 kg KAN ha<sup>-1</sup>) was added at the tillering stage of growth development each season.

Plant samples of each genotype were taken at maturity (five plants per replication). The samples were air – dried and grain yield (GY, g m<sup>-2</sup>), weight of straw at maturity (DMstraw, g m<sup>-2</sup>) and total above – ground biomass at maturity (BY, g m<sup>-2</sup>) were measured. All dry vegetative samples and grain were first ground and then plant N concentration was determined by the standard macro- Kjeldahl procedure. Nitrogen content (at grain, straw and total at maturity) was calculated by multiplying the N concentration by dry weight (gN m<sup>-2</sup>). Moreover, the nitrogen harvest index (NHI) was calculated according to Arduini et al. (2006) as follows:

$$\text{NHI} = \text{N}_{\text{grain}} / \text{N}_{\text{content of aboveground parts at maturity}} (\text{N}_{\text{total}}) (\%)$$

The components of variability, broader - sense heritability, standard errors and coefficients of variability of these parameters, as correlation coefficients, their standard errors and test of significance were determined according to Chaudhary et al. (1999).

### Results and discussion

Analysis of variance (ANOVA) revealed highly significant ( $P < 0.01$ ) differences among genotypes and years as well as highly significant year x genotype interaction NHI. Three-year average of all investigated genotypes was 75% (Table 2). Genotype means for NHI varied from 67% (KG 10) to 79% (KG 165/2).

The N harvest index, defined as N in grain to total N uptake, is an important consideration in cereals. NHI reflects the grain protein content and thus the grain nutritional quality (Hirel et al., 2007). It can be recommended as a selection criterion for nitrogen use efficiency improvement, while improving NUE is one of possibilities for developing new high-yielding quality wheat cultivars (Gorjanovic et al., 2011).

The fact that increasing doses of nitrogen did not lead to increased NHI is very important in terms of ecological agriculture, saving nitrogen fertilizer and minimizing their possible harmful effect on environment. Moreover, the majority of genotypes had the highest value on the control and low N variants (Le Gouis et al., 2000; Chen et al., 2011).

Nitrogen harvest index for wheat usually ranges from 0.70 to 0.80 (Brancourt-Hummel et al., 2003). More than half of studied genotypes had NHI over 75%, which is desirable from a wheat breeding point of view.

Table 2. Average values of nitrogen harvest index (NHI) in three – years investigation

NHI	$\bar{X}$		
Genotype	(%)	S	Cv
Morava	74	1.17	3.64
Lepenica	75	1.63	5.18
Studenica	76	0.75	2.16
Takovcanka	72	0.58	1.82
Toplica	75	1.05	3.05

Srbijanka	71	0.93	2.86
KG – 100	72	0.86	2.76
Lazarica	73	0.86	2.70
Bujna	77	0.51	1.49
Matica	77	0.37	1.10
Vizija	72	0.95	3.26
Pobeda	78	0.73	2.14
Ran 5	76	1.11	3.52
Evropa 90	77	6.22	19/09
Renesansa	77	2.77	8.77
Tiha	74	0.97	3.04
Mina	71	0.71	2.33
Prima	77	1.69	5.00
Kremna	75	1.03	3.37
Rusija	74	0.93	2.98
Pesma	76	1.98	6.41
KG 200/31	74	0.81	2.78
KG 253/4-1	76	0.75	2.41
KG 115/4	77	0.73	2.13
KG 165/2	79	0.73	2.17
KG 56/1	77	0.58	1.78
KG 100/97	76	0.32	1.02
Perla	76	0.93	2.94
KG 224/98	74	0.55	1.70
KG 10	67	1.46	4.97
$\bar{X}$		75	
Factor	A**	B**	A x B**
LSD <sub>0.05</sub>	0.75	2.36	4.09
LSD <sub>0.01</sub>	0.98	3.11	5.38

From a practical point of selection is not desirable that the total variation (phenotypic variation  $V_p$ ) has a greater share of environmental variance ( $V_e$ ) compared to genetic ( $V_g$ ) (Zivanovic, 1997). Significantly larger share of the total variation of genetic variance to the environment means less influence of environmental factors on the realized variation of these traits, which in terms of selection and breeding is considered a very favorable ratio. The obtained results about relation  $V_e : V_g = 7.92 : 25.58$  (Table 3) confirm possibility of use NHI as selection criterion.

Table 3. Components of variability, coefficients of variation and broad – sense heritability of NHI

Trait	year	$V_p$	$V_e$	$V_g$	Relative share of $V_p$ (%)		$CV_p$	$CV_g$	$H^2$
					$V_e$	$V_g$			
NHI	1	22,63	12,19	10,44	24	76	6,63	4,51	0,46
	2	52,10	2,70	22,40			9,19	6,03	0,43
	3	25,76	8,87	16,89			6,81	5,52	0,66
$\bar{X}$		33,50	7,92	16,58			-	-	0,52

The correlation coefficients for NHI and GY (0.65\*\*, 0.49\*\*) and NHI and BY (0.37\*\*, 0.33\*\*) were significant in the second and third year. The relation between NHI and indicators of DM accumulation, utilization and distribution vary depending on the year (Nikolic et al., 2011). NHI is very reliable indicator of nitrogen utilization efficiency for protein synthesis and its correlation with GY depends on the intensity of that process. These results indicate the ability of these genotypes to use nitrogen for protein synthesis more efficiently than for grain filling. This feature may change depending on growing conditions.

Table 4: The simple genotypic and phenotypic correlation coefficients between nitrogen harvest index and grain and biological yield

Indicator	Year	GY	BY
genotypic correlation coefficients			
NHI	1	0,06	- 0.13
	2	0,41**	0.19
	3	0,06	0.07
phenotypic correlation coefficients			
NHI	1	0,10	0.06
	2	0,65**	0.37**
	3	0,49**	0.33**

### Conclusion

Considering obtained results, it can be concluded that investigated materials represents a desirable variability source and has beneficial relationship between variability components and broader – sense heritability. Therefore, it can be considered as important material for future breeding programs of wheat.

Judging by obtained results, NHI, as physiological indicator, could meet requirements to the wheat selection and breeding in terms of improvement productivity in specific circumstances, characteristic, above all, as appropriate safe food production and ecosystem protection.

The strong and statistically high significant interrelationships between grain and biological yield and wheat nitrogen harvest index were registered. The results could be helpful in wheat breeding and for production efficiency while reducing the adverse impacts on the ecosystem. Therefore, the nitrogen harvest index as parameter of wheat nitrogen nutrition efficiency, depending on the circumstances, could be successfully used for the evaluation and selection genotypes, adapted to the low – input systems, ecological and organic agriculture.

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## **YIELD AND QUALITY OF DUAL-PURPOSE BARLEY AND TRITICALE IN A SEMI-ARID ENVIRONMENT IN TUNISIA**

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### **Abstract**

Semi-arid region of Tunisia is characterized by a low and erratic rainfall. This makes year-round maintenance of pasture and forage production under non-irrigated conditions both costly and difficult. In order to fill deficit period, some cereals can be used as dual purpose by cutting or by animal grazing during early stage of growth and then allowed to recover to produce grain. This study aimed at evaluating agronomic performances and grain quality of two dual-purposes cereal crops, Barley and Triticale, cut at the pseudo stem erect stage (C30). The trail was conducted during 2010-2011 season and results have showed that barley yielded more forage crop than triticale without being statistically different, also crude protein in the plant was higher in barley (18.6%) compared to triticale (17.7%). Defoliation has caused a significant grain yield reduction for both cereals and was about 23% for triticale and 33% for barley. Clipping at stem erect stage has a variable effect on different yield components. Thus, higher number of tillers and number of spikes in defoliated compared to undefoliated plants were noted. In the other hand, both number of grains per spike and total kernel weight was negatively affected by clipping.

Grain protein was significantly higher after clipping for barley (11,35% for dual purpose and 10.17% for grain production only) and was not affected for triticale (9.42 % versus 9,57 %).

Under Tunisian semi-arid environment, triticale and barley have comparable yields with a small superiority for barley in forage yield production and higher plant and grain protein contents in triticale.

**Key words:** Triticale, Barley, Forage, Dual purpose, semi-Aride, Tunisia

### **Introduction**

In the north-west of Tunisia, farmers face serious problems of low quantity and quality of forage to feed their animals. This is caused by the poor weather and fertility conditions which characterize the region. One of the solutions, used mainly by local and small farmers, is the practice of cereals dual-purpose. Barley, oat or triticale are grazed or cut in a young stage (tillering) and then allowed to re-growth up to grain production. This will provide forage during winter season which is known as a forage deficit period. Researches about cereals dual-purpose for seeds and forage have given widely varying results according to the climate, to the specie and to the cut or grazing stage. In fact, the practice of first use by cutting or grazing helps to gain a certain amount of nutritious forage, but may reduce straw and grain production particularly when conducted in late growth stage. This practice is common in morocco (Belaird & Morris, 1991); Syria (Mazid & Hallagian, 1983) and Tunisia (Amara et al., 1985). In Mediterranean environments, Hadjichristodoulou (1991) reported that grazing only affected grain yield of dual-purpose barley in rainfed conditions, while the same effect was not detectable in irrigated conditions. Decreases in grain yield after clipping have been

attributed to a reduced number of spikes/m<sup>2</sup> at harvest in barley (Scott et al., 1988) and triticale (Royo et al., 1993) and also a reduced grain number (Bonachela et al., 1995) and kernel weight (Royo et al., 1994). Other studies have reported a grain yield increase after a cutting or grazing during green stage. This Increase has been associated to the decrease of lodging (Droushiotis, 1984). In the other hand; it appears that the management of cutting stage influences the forage and green yield. Royo et al. (1997) have reported that, when cut at the first detectable node stage (C. 31) triticale and barley forage yield was almost double the yield at the pseudo-stem erect stage (C. 30). Defoliation during early growth stages optimizes seed yield and forage quantity and quality (El-Shatnawi et al., 2004). The objective of this study was to investigate the effect of defoliation on production and quality of two dual-purpose species, triticale and barley under rainfall conditions in a semi-arid region of Tunisia.

### **Materials and methods**

The present study was performed in el Kef region, in the experimental field of the higher Institute of Agriculture Kef (36° 11' 9'' N, Longitude 8° 42' 59'' E ; Altitude 652 m). trial was carried out in a a clay-sandy-loamy soil with organic matter content around 1,8%. The climate is mediterranean, with rainfall concentrated in in the autumn and winter. The average annual rainfall is 425 mm on the basis of 50 years.

Two species were used for the experiment : Triticale (*Triticosecale*) variety Tcl 83 and barley (*Hordeum vulgare* L) variety Martin. The experimental design was a Split plot one with 4 replications and each plot mesured 6m<sup>2</sup> in which the main factor was the species and the secondary one the treatment. The two management systems (treatments) experimented were : (i) control plots which were only clipped at seeds maturity in order to estimate the grain yield and (ii) dual\_purposes plots which were clipped first time and harvested as forage at the stem erect stage (C30) and then let regrowth up to seeds maturity. Sowing was carried out early october at a density of 300 viable seeds/m<sup>2</sup>. The soil was chisel plowed in september and right before sowing. Pre-sowing fertilization rates for all plots were 46kg P/ha and 18kg N/ha. During growth cycle 2 fertilizations were provided : 40 Kg N/ha in 3 leafs stage and 50 Kg N/ha after clipping for dual-purpose treatments and in elongation stage for control treatments. Forage harvest was done on 13 February 2011 for barley and 28 February 2011 for triticale. Plants were cut about 12 cm above ground level. Grain harvest was made at seeds maturity on 28 june for both species.

Fresh biomass production from each dual-purpose plot was determined at harvest and it's reported in tones of dry matter (DM) per hectare ; a 500 g sample was also taken for chemical analysis. Forage and grain crude protein content were evaluated by means of the standards micro-kjeldahl procedure. Grain yield, number of spikes/m<sup>2</sup>, number of grains per spike, kernel weight were determined for each plot at seed maturity. Analyses of variance (ANOVA) were performed using SAS (1985) and Means were separated using Duncan Test.

### **Results and discussion**

Total rainfall during 2010-2011 was 685,9 mm (fig 1) which is about 50% more than the annual average (450 mm). A maximum monthly rainfall was registred during december (143mm) and April was a dry month with 40 mm. But because of a wet may (100 mm), dual-purpose triticale and barley grew normally.

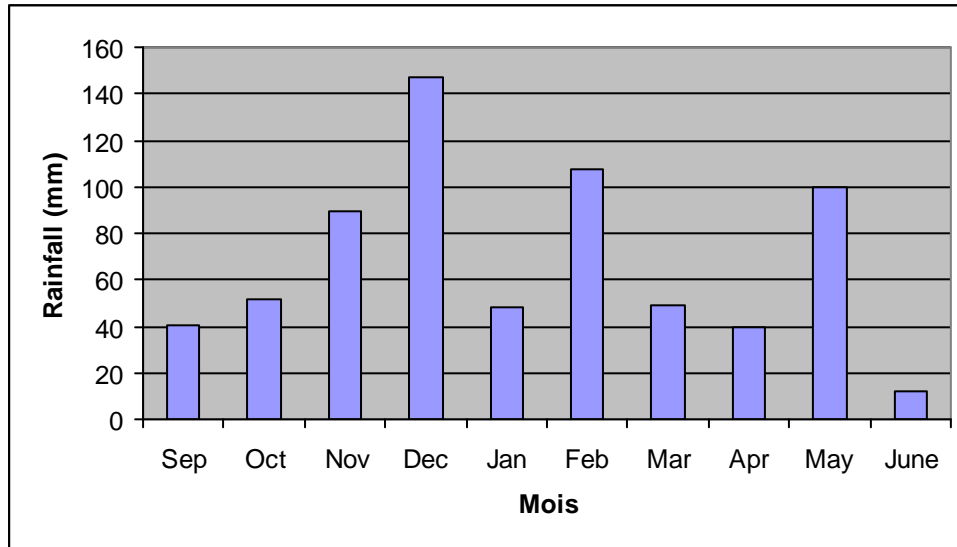


Figure 1. Rainfall distribution for 2010-2011 growing season

### 1. Influence of defoliation at C30 stage and species on grain Yield, components Yield and forage production

Results of forage quantity harvested in C30 are shown in Table 1. Forage yield did not depend on the specie. Barley and triticale has given close quantities of Forage respectively 2,6 t/ha and 2, 4 t/ha. This could be attributed to the fact that the cutting stage is too early that different species could express differences. Specific traits for each specie will be more observable after cutting with regrowth. Royo et al. (1997) have reported, under Mediterranean conditions, similar forage yields in first detectable node stage for triticale (2,03 t/ha) and barley (2,11 t/ha).

The effect of forage removal in barley and triticale on grain yield and its components is shown in table 1. Cutting in green stage (C30) has caused reduction of grain yield for dual-purpose use comparing to grain use only management system. This reduction was more pronounced for barley (-33%) than for triticale (-23%). For both species this reduction was statistically significant ( $p < 0,01$ ). In the other hand, when they were not cut in C30 stage, barley and triticale have given close grain yields which were not statistically different (4,48 t/ha for barley and 4,79 T/ha). For dual-purpose management system, triticale seems to be more adapted and has given a grain yield of 3,69 T/ha which is significantly higher ( $p < 0,05$ ) than barley grain yield (3,01 t/ha). The interaction treatment x specie was significant ( $p < 0,05$ ) for grain yield. Clipping dual-purpose barley and triticale has enhanced spike number production this is explained by the removal of the apical domination during final stage of tillering period. In fact, with defoliation the predominant apex is eliminated and then tillers production restarts again and drive to a higher number of productive tillers by plant. The spike number per  $m^2$  increased from 357 spikes/ $m^2$  to 451 spikes / $m^2$  for barley and from 398 spikes / $m^2$  to 488 spikes/ $m^2$  for triticale. This range of variation was statistically significant ( $p < 0,05$ ). In the other hand, spike fertility was significantly affected only by treatment ( $p < 0,05$ ). In general number of grain/spike has decreased after clipping. The third yield component, 1000 kernel weight, was a very stable variable, being similar for both treatments. It was not affected by forage use neither by the species.

Straw quantity is also important for grain producers in general and for animal keepers in particular as it constitutes an alternative nutrition resource mainly during deficit period. With

this trial, It appear that triticale gives more straw quantity in both managements systems ( $p < 0,01$ ). Clipping was affected significantly straw production ( $p < 0,05$ ) and was reduced by 22% for barley and 26% for triticale.

Table1. Forage yield, grain yield and related components in different managements systems of dual purpose barley and triticale.

	Forage DM (T)	Straw yield (T)	spikes/m <sup>2</sup>	Grain/spike	1000 kernel weight (g)	Grain yield (T/ha)
Barley one use (grain only)	-	8,23 b	387 b	29 a	39,07 a	4,48 a
Barley dual-Purpose	2,6 a	6,37 c	451 a	26 ab	40,18 a	3,01c
Triticale one use (grain only)	-	10,65 a	398b	28 a	41,54 a	4,79a
Triticale dual-Purpose	2,4 a	7,87 cb	488 a	24 b	41,8 a	3,69b
CV (%)	5,76	8,43	7,45	5, 32	4,67	5,76
Species	NS	**	NS	NS	NS	*
Treatment	-	*	*	*	NS	**
Treatment x specie	NS	NS	*	NS	NS	*

## 2. Influence of defoliation at C30 stage and species grain and forage protein content

Table2. Effect of defoliation on grain and forage protein content

	Grain Crude protein (g/kg DM)	Forage Crude protein (g/kg DM)
Barley one use (grain only)	101,76 b	-
Barley dual-Purpose	113,56 a	186,6 a
Triticale one use (grain only)	94,2 c	-
Triticale dual-Purpose	95,7 c	177 b
CV (%)	4,79	6,2
Species	*	**
Treatment	*	-
Treatment x specie	NS	NS

Results of this study indicated that forage barley has a higher protein content (186,6 g/kgDM) than forage triticale (177g/kg DM) when it's cut at C30 stage. Analysis of variance has shown that this difference is significant ( $p < 0,05$ ) (Table2). This is in accordance with Royo et al. (1997) results who have measured around 172g/kg DM for triticale cut in C30 and C31 stages and around 189 g/kgDM for barley cut at same stages.

This experiment has also shown that dual-purpose cultivation affected positively the grain protein content of the two tested species. Clipped plant in C30 stage has given grain more rich in protein: 113,56 g/kg DM Vs 101,76g/kg DM for barley and 95,7 g/kgMS Vs 94,2 g/kgMS for triticale. This increase of grain protein content between treatments was significant only for barley ( $p < 0,05$ ). This significant increase of grain protein after defoliation in C30 stage could be attributed to the dilution effect, since the grain yield was decreasing after clipping and the number of spikes per plant was higher. This conclusion joins results of Francia et al. (2006) who has reported also an increase of grain protein content after clipping during green stage for barley and oat.

### Conclusion

Of the two crops studied triticale demonstrates clear superiority in the grain yield and straw production over barley in the dual-purpose system in the semi-arid region of Tunisia. Triticale has specific morpho-physiological traits that make it more suitable to dual purpose cultivation than barley. Triticale has shown good regrowth after clipping in green stage and reduction of grain yield did not exceed 23%. In the other hand barley has given grain more rich in protein than triticale and this for both management systems.

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**THE EFFECT OF AIR -ASSISTED ON DIFFERENT DOSE APPLICATIONS  
AGAINST SUNN PEST (*EURYGASTER* SPP. HEMIPTERA: SCUTELLERIDAE)  
CONTROL**

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**Abstract**

During chemical applications against plant diseases and pests while some of the pesticide is drifted to non-target area by wind or suspended in the air, the other part drops into non-target sources of water and the ground. As a result of this, negative impacts such as low biological efficiency, high cost and environmental pollution are occurred.

In Türkiye between 2000-2012 which are the years epidemics occurred chemical applications were made against sunn pest at area of approximately 11-18 million da. sunn pest control was conducted by both aerial and ground sprayers until 2002. Since then these applications have been turned into only ground applications gradually. This situation has accelerated national studies that works on increasing the spraying efficiency by reducing spray losses.

The objective of this study was to develop an air-assisted sprayer that is domestic production and suitable for purchasing power of Turkish farmer. This sprayer was evaluated in terms of biological efficiency and distribution uniformity at a domestic wheat variety. The tests were carried out in two stages. Spraying characteristics were determined in the first step. Trials of biological efficiency in which was used Alphacypermethrin EC (100 g/l) at dose of 15.0, 12.5 and 10.0 ml/da against the sunn pest were conducted in the second step (Babaro lu et al. 2013). Thus, efficiency of the air-assisted against sunn pest was brought up to compare with the conventional application and reduction of pesticide use with lower dose application was evaluated. As a result of the study, high biological efficiency was achieved at low doses with air-assisted sprayer.

**Key words:** Air-assisted, sprayer, sunn pest, dose.

**Introduction**

Cereals which are mainly produced in the world are basic food in human and animal nutrition. Besides it is transported and stored easily and also turned into bread and pasta with simple procedures. In Turkey, wheat and barley have the largest production share of the cereals with the planting area of 13.030.000 ha and production of 25.700.000 tons. Cereals that is the most important food material for Turkey are used not only domestic consumption but also exported (Anonymous, 2012/1).

Agricultural production varies from year to year with the effect of various factors. Plant diseases and pests that is one of the most important of these factors cause damage of 15 % in annual agricultural income in agricultural products (Kansu, 1994). Therefore, plant protection against these harmful agents which cause the loss of agricultural product is very important. Both in the world and in our country chemical application is the most widely used control of plant diseases and pests and pesticides are applied with sprayers in this method. However,



during applications all pesticide can not reach the target plant surfaces. while some of them is drifted by wind or suspended in the air, the other part falls into non-target sources of water and the ground. As a result of this, negative impacts such as low biological efficiency, high cost and environmental pollution are occurred. Studies show that 20-25 % of applied pesticide reaches to the target area.

Sunn pest (*Eurygaster* spp.) is one of the major pest which largely causes crop loss in Türkiye. If necessary measures are not provided against the sunn pest, damage may occur up to 100 %. Negative impact of this damage effects yield and quality. Chemical control against the sunn pest has been made in the area of 1 billion hectares per year in Türkiye and insecticide of 150.000 liters has been consumed. This situation has caused a heavy load in the country's economy (Anonymous, 2012/2).

Reducing losses of pesticide, increasing the penetration in both sides of the leaves and protecting environment during applications are necessary for a optimum spraying. However, according to the wind speed and direction uniformity of spray deposition deteriorates, pesticide is collected upper part of plant and doesn't reach under the leaves in applications with conventional sprayers. Thus expected success is not achieved and both environmental pollution and the cost of spraying increase with repeated the applications. It is necessary to use new application techniques for higher biological efficiency by placing pesticide on the the target area and lower spraying costs. One of the application techniques of these is the air-assisted application. The basic aim is to increase spray droplet velocity and modify their trajectory. Air-assisted sprayer appears as the ideal tools to improve the application quality (smaller droplet, in higher number), increase productivity (lower volumes and replenishments, higher displacement speed and extended spraying times), reduce the drift (wind speed of sprayer is greater than the atmospheric wind) and exposure to the products. Result from some studies have shown the potential of air-assisted spraying in providing better coverage and reducing drift by approximately 50 % (Mollrooney at all 1997, Pieche at all 2000).

Air-assisted ground sprayers are not being manufactured in Türkiye. Only a few farmers have imported from other countries and used them. Taking into account all the advantages of these sprayers, an air-assisted sprayer which is suitable for country conditions and purchasing power of farmers was manufactured. Efficiency trials against sunn pest were completed including some low dose applications with Alphacypermethrin.

### **Materials and methods**

The main material of the study consisted of an air-assisted sprayer and wheat field. Trials carried out in two stages. In the first stage, trials were conducted with tracer to determine spraying characteristics in Aksaray-Türkiye. The experimental design was completely randomized plot with three replications. Tartrazine which is a water-soluble food dye was used as tracer and its application rate was 220 g/ha. The cone nozzles which has the plate of 1.2 mm was used at the pressure of 4 bar. Total nozzle flow rate was 16 l/min and application rate was 110 l/ha. Forward speed was 7.8 km/h. Two different air speeds (30 and 20 m/s) were adjusted for the air-assisted applications in the experiments. Filter papers were used as the target surface and placed to the wooden sticks along the plant. Colorimetric method was used to measure the concentration of the tracer. During the applications, average temperature and humidity were measured 26 °C, 33.5 % respectively. Average wind speed was measured 1.6 m/s, 2.6 m/s and 2.5 m/s in the air-assisted applications of 30 m/s and 20 m/s and conventional application respectively.

In the second stage, trials of biological efficiency in which was used Alphacypermethrin EC (100 g/l) at dose of 15.0, 12.5 and 10.0 ml/da against the sunn pest were conducted in Konya-Türkiye. The experimental design was completely randomized block with four replications. Wheat variety of Gerek 79 have the plant height of 40 cm and plant density of 410 number/m<sup>2</sup>. The plot size was about 16x60 m. The number of 12 counting was made with frames of 1 m<sup>2</sup> in every plot on the day before spraying and the first and the third days of after spraying (Babaro lu et al. 2013).

### Results and discussion

The air-assisted sprayer was manufactured by Teknik 20 Mechanical and Electrical Industry and Trade Co. Mean spray deposit of tracer collected on wheat plants (average of the three levels of the plant) for both conventional and air-assisted applications are given in Table 1 and Figure 1.

Table 1. Mean spray deposit of tracer collected on wheat plants

Type of sprayer	Spray deposits of tracer ( $\mu\text{g}/\text{cm}^2$ )
Conventional field sprayer	0.00271 $\pm$ 0.00034 <b>b</b>
Air-assisted field sprayer (20 m/s)	0.00318 $\pm$ 0.00040 <b>a</b>
Air-assisted field sprayer (30 m/s)	0.00325 $\pm$ 0.00040 <b>a</b>

p<0.05

Spray deposits of tracer were higher for the air-assisted applications. Air-assisted application with an air speed of 20 m/s has increased the spray deposits at rate of 17,36 % and with an air speed of 30 m/s at rate of 20,06 % compared to the conventional application (p = 0.014).

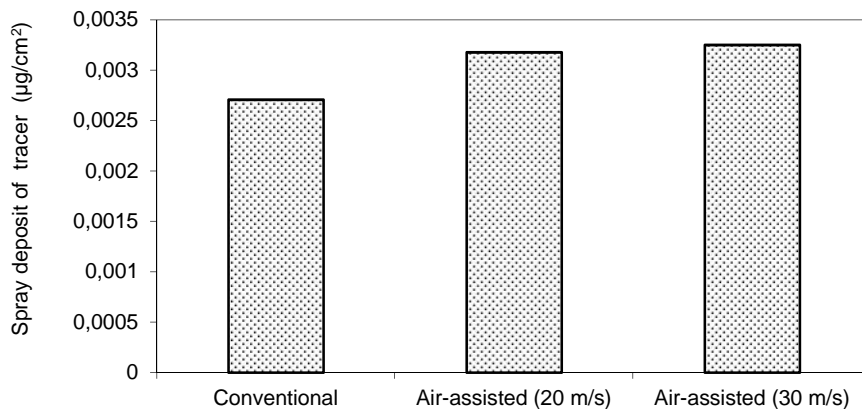


Figure 1. Mean spray deposit of tracer collected on wheat plants

Mean spray deposit of tracer collected on the ground for conventional and two different air-assisted applications were given Table 2.

Table 2. Spray deposit of tracer collected on the ground

Type of Sprayer	Spray deposit of tracer ( $\mu\text{g}/\text{cm}^2$ )
Conventional Field Sprayer	$0.00109 \pm 0.00009$ a
Air-assisted Field Sprayer (20 m/s)	$0.00078 \pm 0.00010$ b
Air-assisted Field Sprayer (30 m/s)	$0.00082 \pm 0.00005$ b

$p < 0.05$

As can be seen in Table 2, spray deposits of tracer collected on the ground in conventional application were measured higher than in air-assisted applications ( $p=0.043$ ). While average of spray deposits of tracer on the ground in conventional application was found  $0.001094 \mu\text{g}/\text{cm}^2$ , in air-assisted applications with air speed of 20 and 30 m/s were found  $0.000780$  and  $0.000823 \mu\text{g}/\text{cm}^2$ , respectively. Spray deposits of tracer collected on the ground in conventional application was higher than air-assisted applications at rate of 40.26 % and 32.93 % respectively (with the air speed of 20 m/s and 30 m/s). In the other words, air-assistance caused reduction of spray deposits of tracer collected on the ground. Spray deposits of tracer collected on the ground increased with raise of air speed from 20 m/s to 30 m/s. Increasing of kinetic energy imparted to droplets with increased air flow velocity caused this situation. Thus the droplets can not hold on to the plant and flow to the ground.

Countings were made to determine nymphs density of Sunn pest (mainly the third and fourth period nymphs) in trials of the biological efficiency on 17/06/2004. Results were given in Table 3.

Table 3. Nymph density of Sunn pest in wheat field in Konya (Babaro lu et al. 2013)

Date	Replication	Control	Density of Sunn pest (number/m <sup>2</sup> )			
			Conventional Field Sprayer 15.0 ml/da	Air-Assisted Field Sprayer (30 m/s)		
				10.0 ml/da	12.5 ml/da	15.0 ml/da
17/06/04	1	16	20	20	23	20
	2	27	37	26	18	26
	3	13	12	27	11	27
	4	21	24	16	21	16
18/06/04	1	22	1	0	0	0
	2	19	2	0	0	0
	3	19	0	0	0	0
	4	24	0	0	0	0
20/06/04	1	18	0	0	0	0
	2	32	0	0	0	0
	3	20	0	0	0	0
	4	26	0	0	0	0

Table 4. Biological efficiencies of the sprayers at first and third days in different doses (Babaro lu et al. 2013)

Type of Sprayer	Dose (ml/da)	Efficiency (%) (Mean)	
		First day	Third day
Conventional Field Sprayer	15.00	97.18	100.00
Air-Assisted Field Sprayer (30 m/s)	15.00	100.00	100.00
	12.50	100.00	100.00
	10.00	100.00	100.00

Biological efficiencies of the sprayers in different doses are shown in Table 4. There were no significant differences between the conventional and air-assisted field sprayers in insecticide applications at full dose of 15 ml/da (0.282 %). Although the dose was reduced at rate of 17 % and 33 % according to the full dose, the effect of 100 % was achieved in spraying with air-assisted field sprayer. Trials of low dose were carried out previously with the conventional field sprayer in a different location and values of biological efficiency remained below of 90 % (84 % and 82 %). Therefore, lower doses were not applied at the trials in the different location due to the small plots. Advantage of the air-assisted application against the conventional application was clear in the spraying conditions which had high wind speeds.

### Conclusion

In our country, the air-assisted spraying technique has been used in orchards for a long time. But for the field plants air-assisted applications are relatively new and especially in the last 10 years the usage of field sprayers has increased. However compared to conventional systems its higher costs impose restrictions on both usage by farmer and manufacturing by firms. On the other hand the increasing in pesticide assembly on target surfaces and higher biological effect with low dose applications can not be excluded. A reduction at a rate of 1/3 of insecticide used against Sunnpest can be provided by this system and it will be extremely important in terms of economy of the country and the safety of environment.

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**THE ADAPTATION OF SOME PERENNIAL RYEGRASS CULTIVARS USED AS TURFGRASS UNDER ANKARA AND ISPARTA CONDITIONS**

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**Abstract**

The efficient use of turf for establishing lawns requires field evaluation of the existing cultivars, especially in the central part of Anatolia with dry climate. Our research was aimed at evaluating the adaptability of the newly introduced and popular perennial ryegrass cultivars in central part of Turkey (Ankara) and in transitional zone of Anatolia to Mediterranean (Isparta). Six perennial ryegrass cultivars (Lucius, Libranco, Lifrance, Eterlou, Taya and Sakini) were seeded with 3 replications in randomized block design in both sites in 2007. Emergency power, establishment potential, winter resistance, cover ratio, leaf texture, leaf color, regrowth potential, tiller number, general appearance, weed invasion and density of each cultivar were evaluated by using a visual score. At the result, there were no really much differences among the cultivars in both sides even that they showed some differences on some parameters in Ankara. All cultivars performed better in summer and autumn than during winter and spring in Ankara. However they were better in spring, summer and autumn in Isparta than during winter period. All of these perennial ryegrass cultivars showed excellent growing, covering, colour, and regrowth after cutting and density in both locations. They were found recommendable for Central Anatolia and transitional zone of Anatolia.

**Key words:** Perennial ryegrass, *Lolium perenne*, Ankara, Isparta, turfgrass, adaptation.

**Introduction**

Perennial ryegrass has a fine to medium leaf texture and tends to be dark green. It is a very competitive cool-season grass, best adapted to coastal regions that have moderate temperatures throughout the year. Grasses vary in tolerance of soil moisture, pH, fertility and temperature ranges. Perennial ryegrass germinates rapidly possesses dark green color, and maintains desirable turfgrass quality during winter months (Allen et al. 1993; Horgan and Yelverton 2001). Perennial ryegrass is often utilized for winter over seeding on golf course fairways and tee boxes, athletic fields, and high profile home lawns. Ryegrass is best adapted to moist, cool environments where temperatures are not extreme in the winter or summer. In Turkey, inland areas are the best adapted for ryegrass. In the transition zone, perennial ryegrass may provide a permanent turfgrass (Avcı et al., 2012). Ankara is in the central Anatolia of Turkey with hot dry summer and with cold rainy winter; however Isparta is in transition zone with milder climate than Ankara. Because of that our objective in this experiment was to test adaptability of newly introduced and popular cultivars of perennial ryegrass (*Lolium perenne* L) under these two different environmental conditions for adaptation.

## Materials and Methods

Perennial ryegrass cultivars (Lucius, Libronco, Lifrance, Taya, Sakini and Eterlou) were used as materials for adaptability testing under Ankara and Isparta conditions. Field experiments were conducted on Ankara University, Agricultural Faculty, Field Crops Department in Ankara, and Süleyman Demirel University, Agricultural Faculty, Field Crops Department in Isparta experimental sites in 2007 and 2008. In Ankara, these cultivars were seeded in a clay loam, classified slightly alkaline (pH 7.8), rich in potassium (1170 kg/ha), poor in phosphorus (69 kg ha<sup>-1</sup>) and containing 1.15% organic matter. In Isparta soil was sandy-loam, slightly alkaline (pH 7.7), rich in potassium (1400 kg ha<sup>-1</sup>), poor in phosphorus (65 kg ha<sup>-1</sup>), and containing 1.10% organic matter and low total salt (0.030 %). According to State Meteorology Department, long-term observations (1975-2010) in Ankara showed that average precipitation, mean temperature and relative humidity were 403 mm year<sup>-1</sup>, 12.1°C and 53.1%, respectively. Corresponding values in 2007 were 310.1 mm, 13.3°C and 59% , however they were 436 mm, 13.7°C and 54.6% in 2008. Isparta showed that average precipitation, mean temperature and relative humidity were 485 mm year<sup>-1</sup>, 12.5°C and 55.7% (1975-2010), respectively. Corresponding values in 2007 were 363.3 mm, 12.68°C and 57% , however they were 463 mm, 13.5°C and 55.8% in 2008.

In Ankara, seeding was done in 12<sup>th</sup> September in 2007, however it was in 3<sup>rd</sup> July in Isparta in 2007 with 50 g m<sup>2</sup> seeds. Randomized block design were used with four replications. Each plot was 2 m<sup>2</sup>. Nitrogen fertilizer as ammonium sulfate was applied as 100 kg ha<sup>-1</sup> with seeding and again in spring of 2008 at both locations. Springer irrigation system was done every day during the summer.

Some observations were done after emergence in both locations by using a visual score (Anonymous, 1991). They are; Emergency power: The number of days between seeding (planting) days and when 50 % emergence was occurred in plots. Covering potential: The number of days between seeding (planting) date and when 75% plants were seen in the plot. Winter resistance (1-9): Observations were done at the beginning of spring growth which was at the end of February (1=very bad (dead of all plants), 3=bad (50% plants died), 5=medium (all plants of the plot were yellowish), 7=good (less than 50% was yellowish) and 9=well (no yellow plant in plot). Leaf texture (1-9): Leaf width on leaves was measured (1=very rough (more than 4 mm), 3=rough (3-4 mm), 5=medium (2-3 mm), 7=fine (1-2 mm) and 9=very fine (less than 1 mm). Leaf colour (1-9): Observations were done in spring, summer, autumn and winter (1=yellow, 3=light yellow-green, 5= green, 7= dark green and 9=very dark green). Regrowth potential (1-5): In spring, before second cut regrowth potential was observed (1=high regrowth, 3=medium regrowth and 5=low regrowth). General appearance (1-9): Plots were observed for general uniformity, colour, tissue, liveliness, weeds, disease and insects in each season (for spring, summer, autumn and winter) (1=very bad , 3=bad, 5=medium, 7=good and 9=well). Weed invasion (1-5): In second year, after last cut at the end of vegetation, the weed invasion on plots was observed (1=high, 3= medium and 5= no weeds in plots). Density: In second year at the end of vegetation stage, the plant density was observed in each plot (1=very rarely, 3=rarely, 5=medium, 7=dense and 9 = very dense).

The data were analyzed by GLM at the 5 and 1 % levels of significance. When a significant differences was found, a protected Duncan test was applied at the p=0.05 level for comparisons between means (SAS, 1985).

## Results and discussion

The data of both experiments (in Ankara and Isparta) of perennial ryegrass cultivars used as turfgrass were analyzed and the Duncan results were given in Table 1 and 2. In Ankara, there

were significant differences among the cultivars on emergency power ( $p<0.01$ ), leaf texture ( $p<0.05$ ), leaf colour on winter ( $p<0.05$ ), spring ( $p<0.01$ ), summer ( $p<0.05$ ) and autumn ( $p<0.05$ ), regrowth potential ( $p<0.01$ ), general appearance on summer ( $p<0.05$ ) and density ( $p<0.01$ ). However, there were no differences between cultivars on covering potential, winter resistance, and general appearance on winter, spring and autumn and weed invasion. Just the opposite, only two observations (leaf texture and density ) showed the significant difference among the cultivars in Isparta. All other observations did not make any differences among the ryegrass cultivars in that area.

According to the research, some observations of turfgrass measurements were differed for each perennial ryegrass variety under Ankara conditions. Some cultivar showed better performance than others for each observation apart. In Isparta they did not show any differences in any observation (Table 1 and 2). Cultivars showed very close results and plots were similar to each other. As the result, there were no really much differences among the cultivars in both sites. Their germination, emergency and covering capacity were very fast. All cultivars performed better in summer and autumn than during winter and spring period in Ankara. However they were better in spring, summer and autumn in Isparta then during winter, because Isparta is in transitional zone of central Anatolia with more temperate climate than Ankara. According to Richardson (2004) the annual and intermediate cultivars of ryegrass all showed increased high-temperature stress under increasing temperatures comparing to the perennial cultivars, which did not show stress until air temperature exceeded 40°C. Sampaux et al. (2013) found that turf winter greenness had been marginally improved, whereas summer greenness and seed yield had not been significantly changed. Same researcher also said that turf density and fineness played a major role in the visual assessment of turf aesthetic merit and that wear tolerance was closely associated with turf density. Similar to this, all of these perennial ryegrass cultivars in our research showed excellent growing, covering, colour, and regrowth after cutting and density at both locations with high temperature during the hot and dry summer. Opposite to the our research, Kır et al. (2010) had trials in Mediterranean environment with perennial ryegrass cultivars. They performed better in winter, spring and autumn than during summer period. These results showed that environmental conditions especially climate were playing an important role in growing perennial ryegrass and its turfgrass quality.

Perennial ryegrass is generally used where rapid establishment is a necessary: for home lawns, parks, golf course fairways and roughs, airfields and -- especially -- athletic fields. It is a medium-textured turf with good shoot density and uniformity that is frequently over seeded into warm-season turfgrasses in the winter months to supply a dark green color in warmer climates (Anonymous, 1995).



Table 1. Perennial ryegrass (*Lolium perenne*) cultivars (ANKARA)

Cultivars	Emergence power	Covering potential	Winter resistance	Leaf texture	Leaf colour				Regrowth potential	General appearance				Weed invasion	Density
					Winter	Spring	Summer	Autumn		Winter	Spring	Summer	Autumn		
Lucius	6.25 b*	16.0	6.50	4.00a	4.50ab	5.00cd	7.00b	6.50b	3.00a	7.0	9.0	9.00a	9.0	5.0	9.00a
Libronco	6.50 b	16.0	6.00	3.00ab	4.00b	7.00ab	7.00b	9.00a	3.00a	7.0	9.0	9.00a	9.0	5.0	9.00a
Lifrance	6.50 b	16.0	6.00	3.50ab	4.50ab	6.00abc	8.00ab	8.50a	3.00a	7.0	9.0	9.00a	9.0	5.0	8.50a
Taya	9.00 a	16.0	6.50	2.00b	4.50ab	7.50a	8.50a	8.0ab	2.00b	7.0	9.0	9.00a	9.0	5.0	9.00a
Sakini	7.25 b	16.0	6.50	2.50ab	4.50ab	4.00d	7.00b	6.50b	1.50b	7.0	9.0	8.00b	9.0	5.0	7.00b
Eterlou	7.25 b	16.0	6.50	4.00a	5.00a	5.50bcd	8.00ab	8.0ab	3.00a	7.0	9.0	9.00a	9.0	5.0	9.00a
Mean	7.13	16.0	6.33	3.16	4.50	5.83	7.58	7.75	2.58	7.0	9.0	8.83	9.0	5.0	8.58
C.V.	14.9		13.31	31.2	12.39	16.95	11.03	12.97	22.71			5.33			4.75

\*Means of each populations in a column followed by the same lower case letters are not significantly different ( $p < 0.05$ )

 Table 2. Perennial ryegrass (*Lolium perenne*) cultivars (ISPARTA)

Cultivars	Emergence power	Covering potential	Winter resistance	Leaf texture	Leaf colour				Regrowth potential	General appearance				Weed invasion	Density
					Winter	Spring	Summer	Autumn		Winter	Spring	Summer	Autumn		
Lucius	8.25	10.50	5.00	7.00a*	2.50	6.50	5.50	6.50	1.0	6.50	7.50	7.50	6.50	5.00	9.00a
Libronco	8.25	10.50	5.00	5.00b	2.00	6.00	6.50	7.00	1.0	6.50	8.00	8.50	7.00	5.00	8.50ab
Lifrance	8.50	11.00	5.00	6.50a	2.50	6.50	3.00	6.50	1.0	6.50	8.00	8.00	7.00	5.00	9.00a
Taya	9.00	11.25	4.50	7.00a	2.00	6.00	6.50	7.00	1.0	6.50	8.00	8.00	6.50	4.50	7.50b
Sakini	8.50	10.75	4.50	7.00a	2.00	5.50	6.50	6.50	1.0	6.50	8.00	8.00	6.50	4.50	7.50b
Eterlou	9.00	11.25	4.50	7.00a	2.00	6.50	6.50	7.00	1.0	6.00	8.00	8.00	7.00	4.50	8.00b
Mean	8.58	10.87	4.75	6.58	2.16	6.16	6.25	6.75	1.0	6.42	7.92	8.00	6.75	4.75	8.25
C.V.	9.51	7.67	14.55	6.20	25.63	10.25	20.72	8.11		14.6	12.4	13.69	11.2	14.6	11.07

\*Means of each populations in a column followed by the same lower case letters are not significantly different ( $p < 0.05$ ).

## Conclusion

Perennial ryegrass cultivars were grown in Ankara and Isparta as turfgrass to determine their adaptation during 2007 and 2008. According to the results, there were no significant differences among the cultivars in both sides even that they showed some differences in some parameters in Ankara. All cultivars performed better in summer and autumn than during winter and spring period in Ankara. However they were better in spring, summer and autumn in Isparta then during winter. All of these perennial ryegrass cultivars showed fast germination, emergency, covering potential and excellent growing, colour, regrowth after cutting and density in both locations. They were found recommendable for Central Anatolia and transitional zone of Anatolia.

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## QUALITY OF ROTARY MOWER SIP RK 135 MOWING PROCESS IN MOUNTAINOUS AREA

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### Abstract

The paper presents research results of tractor rotary mowers SIP RK 135 in cutting the first swath in natural landmeadow. The average yield of green mass was 20.66 t/ha. The aim of this research was to determine the productivity, quality of work and losses using the tractor rotary mower in cutting the first cut landmeadow. In the first experiment tractor operated at an average speed of 5.89 km/h achieving performance of 0.69 ha/h. In the second experiment the tractor operated at an average speed of 9.29 km/h achieving a performance of 1.01 ha/h. At a given speed, efficiency of work operations ranged from 0.78 to 0.93, averaging 0.86 in the first version and 0.70 to 0.93, averaging 0.81 in the second version. With the increasing speed of operation it has been observed an increase in the average height of cut by 1.35 cm and also increase in total losses of 2.73 % to a maximum of 4.45 % of the total yield.

**Keywords:** rotary mower, mowing, operational productivity, cutting height, losses.

### Introduction

Mowing is the first work operation - phase in the procedure of storage of the hay, green forage and silage. Mowing is performed as a separate work operation or in combination with other work operations such as crushing or conditioning. Mowing of the biomass is performed at the time when the plant contains the most nutrients in order to preserve the value of the biomass and mowing should be done in the shortest possible time. The machines that are used for mowing grass should meet several general requirements, such as:

- universal use;
- cleanliness of the cut;
- prevention of pollution of biomass with soil;
- high operating speed - efficiency;
- easy, simple maintenance - technical and aging;
- work with minimal losses.

Almost all types of tractors and mowers meet the above requirements. In storing of hay obtained from meadowland the aim is to absorb as much biological yield of green mass, among other things, in order to reduce losses. In alfalfa in order to prevent damage to the cluster optimum cutting height is considered to be 6 to 8 cm, ie. it should not be mowed under 6 cm, while in the natural meadowland this height is somewhat lower up to 4 cm, which in turn depends on how the parcel is leveled plot and floristic composition of natural meadows. Today, in practice most commonly used are two types of tractor mower, that is oscillating and rotating. No matter what the mowers are being used in mowing losses arise. Losses may arise due to the large height of cut and as a result of fragmentation of mowed mass, which during handling the hay can not be collected, but remain on the land. If we bear in mind that the greatest amount of chopped mass consists of leaves containing the highest amount of

nutrients, thus a special attention must be paid to this type of loss. The speed of mower affects the increase in losses. (Tanevski, 1990), in examining the self-propelled mowers-hay crusher, quoted the average speed of 5.35 km/h. The same author states that the efficiency of work procedures amounts to average 95.5% of the yield. With the increasing speed of the mower an increase in losses due to cutting height occurs, but there is also reduction in losses that occur as a result of fragmentation. (Gašparac, 1988) in examining the SIP 165 rotary mowers at speeds from 8,5-12 km/h achieved the cutting height from 4.30 to 6.28 cm and width of 132-154 cm. According to Potkonjak (1986) the self-propelled mower "Fortschritt" substantially deviated from the optimal height adjustment. Deviation amounted to 9.47 cm, and consequently an increase in alfalfa mass loss in cutting height, with an average of 1.63% of yield. Koprivica (1996) in examining the self-propelled mowers and hay crusher in mowing alfalfa quoted the speed of the mower in the range of 3.71 to 6.41 km/h. To prevent damage to the cluster, optimum cutting height of 6 - 8 cm is considered to be optimal for alfalfa, which can be accomplished at working speeds of 5-8 km/h for conventional mower, and for the rotational 8-15 km/h (Zoranovi, 1995). According to Zoranovi and Potkonjak (1996), the total losses when mowing varied in the range from 0.90 to 2.29% of the yield, i.e. at average 1.59% of the yield. Height of cutting was the 6.42 cm at average. As recommended by Wiersma and Wiederholt (2001), alfalfa should be mowed to a height of 2 inches (5.08 cm). Potkonjak et al. (2009), suggest that the classical mowers accomplish the most favorable cutting height, as they better maintain the set cut-off level. According to the same authors classical mower has incurred the lowest total losses (approximately 1.27% of the green mass yield). Rotary mowers have achieved higher overall losses, i.e. mower with 6 discs of 3.16%, and 2.75% with 14 disks. Vukovic (2009) states that, with the increase of movement speed mower working width is reduced, which results in an increase in total losses. Also, the losses incurred due to cutting height increase, and losses incurred due to fragmentation decrease. Total losses recorded in the classic cutting apparatus amounted to 1.18% of the yield of alfalfa, while in self-propelled by 1.52% and 2.99% in rotary mower, says the same author. Losses of the mowing should not exceed 5%. The accomplished productivity varied in the range of 0.72 ha/h (oscillatory) to 1.00 ha/h (rotary mower). (Vukovic et al., 2011). According to researches performed by Bara (2012) the lowest cutting height was observed in rotary mowers with the drums and was 5.83 cm, with an average operating speed of 9.53 km/h. The minimum cutting height of the mower was measured at the velocity of 8.40 km/h and was 5.19 cm, and the maximum one of 6.48 cm at a speed of 10.84 km/h.

Mower with classic cutting apparatus accomplished a slightly higher cutting height in relation to the rotary, and it averaged 6.32 cm, with the tractor average operating speed of 5.79 km/h. The lowest cutting height of 5.42 cm was at 3.72 km/h, and the maximum speed of the tractor of 8.23 km/h was 7.38 cm.

According to research of Sila-Bogosavljev (1985 drawn mower Dubrava, Gramip TSK Superior, at working speed of 6.0 to 14.8 km/h achieved the effect of 0.81 to 2.00 ha/h, the coefficient of utilization of constructional working width is 89-97%, and the cutting height varied from 7.0 to 8.6 cm.

### Materials and method

Experiments were carried out on the family farm in Mokro, near Pale, when mowing natural meadow with tractor rotary mower with drums SIP RK-135, at parcel with an average yield of 20.66 t/ha. Green mass yield was determined by measuring the green mass of meadow in the length of one meter of mowing width, calculated per hectare. The mower speed is determined using chronometric method. Using hand-held stopwatch the speed is measured on the length of 50 m. The cutting height is determined at the point where the loss is being determined, in such way that, for each test on the corresponding surface, measured was height of all stubbles on the basis of which the average was determined. Losses during mowing, measured from the surface of one meter long swath, at the working width of the mower, at the same place where the cutting height is determined. Total losses are presented as the sum of losses incurred due to cut height and losses incurred due to fragmentation. Determination of losses was performed in four tests.

### Results and Discussion

Rear side drum rotary mower SIP RK 135 is one of the classical mower intended for small and medium-sized farms. They are characterized by simple construction and transmission of power to drive the drums through the belts and gears in the carrier housing. Rotary mower SIP RK 135 cuts grass mass on the principle of free cutting. This mower has a vertical drums or cylinders, with the cutting blades at the bottom. Drums or cylinders are placed in such position so that they work in pairs, and both drums have 3 blades.

Table 1. Technical characteristic of rotary mowers SIP RK 135

Parameter	Unit	Value
Suspension Point Category	-	I + II
Working width	m	1,35
Weight	kg	325
Transport width	m	1,20
Vehicle length	m	2,50
PTO Shaft rpm	min <sup>-1</sup>	540
Drum rotation rpm	min <sup>-1</sup>	2250
Number of drums	-	2
Number of knives	-	6
Required tractor power	kW	18
Cutting height	mm	40-70
Mowing capacity	ha/h	1,2

In the course of operation drums have opposite directions of rotation one towards other in pairs when moving, blades are cutting off the biomass and dispose it in swath between the two drums.

Researches were carried out on parcels of natural meadowland in the first swath. The average yield of green mass was 20.66 t/ha and varied in the range from 17.95 to 27.06 t/ha. Height of grass mass ranged from 50 to 140 cm, on average 87.36 cm. Technical characteristics of the rotary mowers SIP RK 135 are given in Table 1. Side tractor rotary mower SIP RK 135, supported at three points, represents standard equipment for mowing primarily on small and medium-sized farms. The model which was tested has two rotating drums driven by pair of gears. They are equipped with safety device and the bracket connection of the cutter bar for

unhooking. This mower is characterized by a simple but strong construction and exceptional durability.

During testing rotary mower SIP RK 135 was combined with the tractor IMT 540 Deluxe. In the first test, the tractor while moving in first gear achieved an average speed of 5.89 km/h with a CV of 3.11%, while in the second test, while moving in the second speed gear achieved an average speed of 9.29 km/h with a CV of 11.45%. (Table 2).

Mower constructive working width is 1.35 m and during testing efficiency of work procedures varied from 0.78 to 0.93, on average of 0.81 of the constructive, whereas in the second version observed was a tendency to reduce the coefficient of utilization of working width with increasing speed and is 0.70 to 0.93, average of 0.81.

Table 2. Operation Productivity SIP RK 135

Parameters	Speed I			Speed II		
	Average	SD	CV%	Average	SD	CV%
Operating speed (km/h)	5,89	0,18	3,11	9,29	0,58	6,24
Working width (cm)	116,5	6,42	5,51	108,8	8,97	8,24
The coefficient of utilization of working width	0,86	0,05	5,51	0,81	0,07	8,24
Production capacity (ha/h)	0,69	0,04	5,99	1,01	0,12	11,45

Table 3. Stem cutting height (cm)

Mower type	Parameter	Speed I	Speed II	Average
SIP RK 135	Cut-off height (cm)	5,15	6,50	5,83
	Speed (km/h)	5,89	9,29	7,59

Optimum height of alfalfa cut off, to prevent damage to the cluster, is considered cutting height to be from 6 to 8 cm, i.e. it should not be cut off under 6 cm, while in the natural meadowland this height is somewhat lower up to 4 cm and even lower, which in turn depends on how flat the surface of the parcel is and on floristic composition of natural meadowland. The results presented in Table 3 indicate that the change of moving speed has a significant impact on the cutting height of the tested mower. The average height of cutting of stems was 5.83 cm at an average speed of 7.59 km/h, the minimum cut-off height was 5.15 cm while moving in first gear at a speed of 5.89 km/h and the maximum height of cut-off was 6.50 cm at a speed of 9.29 km/h.

Table 4. Operating losses (% of yield)

Mower Type	Type of loss	I speed	II speed	Average
SIP RK 135	Cutting height incurred losses	1,67	3,63	2,65
	Losses due to fragmentation	1,06	0,82	0,94
	Total losses	2,73	4,45	3,59

It has been noticed on the mover which is being tested that, with increase of the moving speed, there is a tendency to increase losses due to cutting height and to decrease losses due to fragmentation. The results presented in Table 4 suggest that losses due to height of cut with rotary mowers varied depending on the speed in the range of 1.67 to 3.63 %, at average of 2.65%, and the losses due to fragmentation were ranged from 0.82 to 1.06 % at average of 0.94 %. The total losses incurred are the sum of the previous two types of losses and during the testing of rotary mowers average value of total losses amounted to 3.59 % of yield, which is in accordance with other authors who have dealt with this issue.

## Conclusion

Based on the results of testing the rotary drum mowers SIP RK 135 quality of cutting we can say that the same achieved optimum and uniform losses in tested variants. Thus, the lowest cutting height of 5.15 cm at speed of 5.89 km/h and with increasing speed of 9.29 km/h it was 6.50 cm. Cut-off heights of 4 cm in the meadowland depends solely on how flat the surface of the parcel is, condition of the and to which extent the land is weedy. SIP RK 135 has made losses incurred by height adjustment of 2.65 %, whereas the losses due to fragmentation decreased with increasing speed of 1.06 to 0.82 % and the total average losses were 3.59 %. If we bear in mind that the maximum allowed value of losses is 5 % of the yield, it can be concluded that the rotary mower SIP RK 135 has achieved the optimum value of losses and quality of mowing.

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## BREEDING FOR CROP IMPROVEMENT

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### Abstract

In a frame of plant breeding are developed different methodologies directed towards genotypes improvement. Thousand years ago, a man practiced selection through domestication, cultivation and production plants that have more desirable traits than wild plants. Cultivated crop species selected from wild populations is called a landrace. Creation of new cultivars has been done by using of simply plants selection techniques choosing desirable characteristics for propagation, to more complex molecular techniques. Conventional breeding is based on homologous recombination between chromosomes to generate genetic diversity. Also, breeders may use a number of *in vitro* techniques such as protoplast fusion, embryo rescue or mutagenesis to generate diversity and produce hybrid plants that does not exist in nature. Breeders have the task to incorporate into crop plants improved traits: quality and yield, tolerance to salinity, extreme temperatures, drought, resistance to viruses, fungi and bacteria, increased tolerance to insect pests and herbicides. The most cultivars are created by crossing two parents. Created cultivars have changes of architecture, ripening time, productivity. Soil moisture is the most limiting factor in dry land agriculture. It is lost as evaporation from the soil surface and as transpiration from the plant surfaces. Technology growing and soil fertilization related to productivity of plants. The evaporation losses can be reduced by mulches, antitranspirants, wind breaks, weed control. In the coming future with climatic changes are necessary protected wild relative species and other existing genetic resources in nature and gene bank for successful breeding.

**Keywords:** Breeding, crop, productivity, genetic resources, improving adaptability

### Introduction

During the practice, the man is permanently treed to make changes in nature, structure of plants and their functional properties in the aim to provide for enough food. Since near the beginning of human civilization, by simple selection of plants, fruits and seeds man had the greatest benefit from cultivated plants. Man is learned from nature how to develop new and new approach for environmental protection, improvement and cultivation of crop species. That was base for developed efficient techniques and methods as well as developing of plant breeding. In the breeding practice man used heterosis in plants, induced mutations, and nowadays new techniques in biotechnology (Knezevic et al., 2012). From the beginning of manipulation with plants, man used from nature the superior cultivars according to yield and quality of fruit, seed, biomass of vegetative organs and resistance to environmental stress factors. These natural resources of plant species, man firstly use for: a) domestication and later for b) classical plant breeding, c) modern plant breeding as well as for d) genetic modification.

Within domestication of plant, which approximately started 10 thousand years ago, man selected and produced plants with more desirable plants. Numerous crops are cultivated today

as a results of domestication of plants (wild relatives, landraces) in different period (neolith) in past in different region of Old World (about 5 thousand years ago) and later in region of New World (about 3 thousand years ago). Generally we can say that plant crops for food originated from domesticated plant in ancient time. These crops characterize adopted morpho-physiological traits (resistant to lodging, resistance to pests and disease, tolerance to abiotic stress factors, pollination and ripening time) high productivity, quality and genetic diversity.

### **Plant breeding approaches**

Classical plant breeding started by crossing of different individual plants in the aim of developing new crop or cultivar. On this way produced ofsprings possess mixed genetic material from both parents. For increasing yield in the practice obtained ofsprings crossed with the high yielding parent. Also, plants may be crossed with themselves to produce inbred cultivars for breeding. Produced ofsprings use for testing of productive, physiological traits and quality properties. Recombination of genes generates genetic diversity within created plant population. However, breeder can use *in vitro* techniques such as protoplast fusion, embryo rescue or mutagenesis to generate diversity and efficient creating cultivar or hybrids that would not exist in nature. Application of breeding methods contributed to improvement of agro economic traits of plant species. Developed cultivars and hybrids characterize increasing of yield in average 1% annually. Also, by classical breeding in the previous century improved quality of the crop, resistance to fungi, viruses and bacteria diseases, increased tolerance to insect pests, environmental stress factors (drought, frost, salinity) and tolerance to herbicides. Changes in characteristics of the plants in the cross breeding process have been carried out on the basis of reproductive compatibility (Kondic et al., 2012) and had a contribution to maintenance and increment of genetic variability and economic impact in terms of increasing the quantity and quality of food (Živan ev et al., 2012). Modern plant breeding use techniques of molecular biology that has converted classical plant breeding to molecular plant breeding. By using of molecular biology methods improved precise insertion and manipulation of genes controlling desirable traits. In modern plant breeding used marker assisted selection or marker aided selection (MAS) that is approach of a marker morphological, biochemical or one based on DNA/RNA variation. These markers are used for indirect selection of a genetic determinant of a desirable trait (productive components, quality properties, resistance to disease and tolerance to abiotic stress).

Natural resources used in breeding program of genetic improvement ( uric 2009). Sufficient methods of molecular biology make possible modification of plants by adding or delete specific genes from evolutionary unrelated species. Plants produced on this way are named as transgenic plants. By this methods is possible create plant with the desired traits faster than by classical breeding because the majority of the plant's genome is not altered. The majority of commercially released transgenic plants, carried genes controlling resistance to insect pests and herbicides. For example Insect resistant plants created by transferring a gene from *Bacillus thuringiensis* (*Bt*) that encodes a protein that is toxic to some insects. Herbicides usually work by binding to certain plant enzymes (known as the herbicides *target site*) and inhibiting their action. In the case of herbicide resistance crops with achieved a version of target site protein that is not inhibited by the herbicide were produced glyphosate resistant crop plants. The new step in genetic modification is creation plants that can produce pharmaceuticals and industrial chemicals. This represents new area of breeding which sometimes called pharmacrops. All produced transgenic plants and genetically modified organisms are in focus of discussion, question and dilemma of scientist, politicians, consumers, producers, salvers, public media wide and specific population (Knezevic *et al.*, 2012). In spite that GM plant have significantly increased production the negative effect of

GM plants are reflected in the appearance of loss of genetic diversity of crops, change the nutritional quality of food, appearance of potential monopoly ownership of major food products and sometimes lead to non safe consumption.

### **Criteria and plant species in focus of breeding**

As we told, cereal crops, domesticated from wild grasses thousands of years ago. Domestication of the cereals, maize (*Zea mays*), rice (*Oryza sativa*), and wheat (*Triticum spp.*), are conducted in different regions of the world 7,000 to 10,000 years ago. The main three food crops are selected with much higher annual production than others are. Also, the barley (*Hordeum vulgare*), is among the earliest domesticated cereals as the fourth important cereal crop used as human food, animal feed, and brewing grains. Considering the annually quantity of production the next important cereal is the Sorghum, oat (*Avena sativa*), rye (*Secale cereale*), and millets. The completion of rice genome sequencing has greatly accelerated knowledge about rice domestication and origin (Kovach et al., 2007; Sweeney and McCouch, 2007; Vaughan et al., 2008). Among the cereals, maize differs from barley, rice, and wheat by having a lot of different morphological modifications during domestication (Doebley et al., 2006). The high progress in yield increase achieved in barley, rice, and wheat, through breeding improvement of phenotypic traits that allowed effective harvesting, such as reduction in shattering and improvement of threshing, enhanced quality.

Selection on phenotype is a powerful approach to realize directed desirable changes requires genetic variability of potential parent for crosses and long time of selection. For the successful breeding is important to know how many genes and mutations were enough for the crop improvement through domestication. The aim of breeders is to provide methods and materials for breeding for high productivity, resistance to stresses and specific demands on the quality of products, as well as for effective response to growing conditions. Utilization of genetic resources, as donors of important characters and creation of initial breeding materials also contribute to the improvement of biological potential of crops and broadening of their genetic diversity (Uric 2009). Breeders are used identified effect of mutations, example, a single mutation controls primarily no shattering in all rice cultivars, free threshing or naked seeds in barley, and naked grains of maize *gal* (Wang et al., 2005). Also, used genes with different mutations for the origin of white seeds of rice and six-rowed ears of barley. In maize, *tb1* was responsible for the reduction of lateral branches of the wild progenitor (Doebley et al., 1997). For the efficient breeding is very important to know whether a single mutation or multiple mutations of independent origins were involved in this domestication transition (Clark et al., 2006). The location of genes and other markers in DNA fragments is necessary determine to make physical maps of genes along a chromosome. Physical maps are important for gene cloning, the development of genetic markers for tagging genes during breeding.

Simultaneously, increased attention is paid to the conservation and utilization of genetic resources as irreplaceable sources of genes for further genetic improvement of crops. To meet all these demands intensive research on genetics, biotechnology, breeding techniques, genetic resources and seed technologies is needed.

Conventional breeding can manipulate multiple traits simultaneously and can manipulate genetically complex “quantitative traits” i.e. traits that are influenced by the environment and traits that are conditioned by multiple genes. Advances in wheat genetics and genomics are essential for the sustained development of wheat cultivars with increased yield potential, resistance to pests and diseases, and tolerance of adverse environmental conditions. Wheat is also polyploid; it originated by interspecific hybridization, and its nucleus contains three different genomes, designated A, B, and D, each originated from a different diploid species.

Scientists must have access to genetic diversity to help create new cultivars or hybrids that can resist pests, diseases, and environmental stresses and need to know molecular base of breeding (Karp et al., 1997). The success of breeding depends from existing divergent crop germplasm, level of its preserving and evaluation, as well ability of distributing crop germplasm. The broad potential of recombinant DNA technology will provide the possibility of both molecular analyses of crop productivity and ways in which it may be possible to improve that productivity (Mifflin, 2000). The research may be approached in different ways: a) by generating complete sequences of the plant genome, b) by genetic analysis of phenotypes using genetic marker technology and c) by metabolic analysis.

The developments in molecular genetics in wheat have been relatively slow in comparison to other crops, such as maize, rice or tomato, due to wheat's ploidy level and complexity of its genome, percentage of repetitive sequences and level of polymorphism. Much fewer maps exist in wheat and far fewer QTL studies have been reported when compared to other grass species.

However, due to the large number of disease and pest resistances controlled by major genes, the mapping of such genes has dominated the research activities in wheat molecular genetics. The hexaploid nature of wheat and its amenity to cytogenetic manipulation have offered unique tools for molecular geneticists of wheat. These include the use of various aneuploid stocks, such as nullitetrasonic and ditelosomic lines, to assign molecular markers to specific chromosome arms (Anderson *et al.*, 1992; Plaschke *et al.*, 1996), of chromosomal deletion stocks (Endo and Gill, 1996) for the physical mapping of markers (Röder *et al.*, 1998) and of single chromosome substitution lines to map genes of known chromosomal location (e.g. Galiba *et al.*, 1995; Peña *et al.*, 1997).

### **Breeding for adaptation to environment**

Classical methodology of breeding also follows new trends in research an application of new techniques. This approach will contribute to the making progress of theoretical and methodological bases for applied research and breeding and to provide new developed materials for genetic improvement of agricultural crops. Such cultivars are also the most effective tool how to minimize negative impacts of agriculture on the environment. Among all inputs in agricultural systems, the genetic improvement of crops can be considered as the most effective way of increasing plant production and quality without significant increase in costs and negative effects on the environment. Specific combination of environment condition have influence to growth development and differed depends of crops (Micic, 1996). In addition, breeding for resistance to pests, diseases and abiotic stresses as well as for effective utilization of nutrients and effective response to growing conditions can lead to decreased demands on other inputs, especially inputs of agrochemicals. (Paunovic et al., 2007).

A wider genetic diversity of crops and cultivars contributes to more stable production and its quality in balanced agro ecosystems. Nowadays, achieved progress in molecular and cell biology, *in vitro* techniques as well as applied genetics and breeding methods speed up further progress in agriculture through new cultivars and technologies. The breeding of crops and other plant species directed to verify methods for selection of genotypes with high efficiency of water use in dry conditions concerning the effects on transpiration and photosynthetic assimilation of flag leaves. The tested winter wheat cultivars could be divided into four groups according to their water use efficiency in dry and wet conditions: 1) genotypes economized water in both dry and wet conditions, 2) economized water only during water stress, 3) wasted water no matter whether there was a water deficit or a water surplus, and 4) genotypes squandered water even when there was its deficit. The second group could be important for breeding.

Potato genotypes sensitive to water stress were found to differ significantly in the effectiveness of water utilization in both variants, in wet and dry conditions.

Drought-stressed leaves spring barley cultivars showed a significant drop in leaf conductance and a reduction of photosynthetic rate. The root system of winter wheat varieties is markedly more sensitive to water deficit in comparison with the root system in wet conditions (Dodig et al., 2007). For this study successfully can use methods based on gas analysis used to monitor the efficiency of water use in leaves showed high accuracy with high sensitivity to physiological changes of leaves during water stress. Anatomical and morphological studies can provide important data to complete the explanation of physiological responses of leaves and root systems to water deficit (Micic, 2009).

One of the criteria of breeding is to find the relationship between vernalization requirements and dynamics of frost tolerance in wheat cultivars and lines with substitutions of homoeologous group 5 chromosomes carrying vernalization genes (*vrn*). By crossing the substitutions of dominant genes *Vrn* from spring to winter cultivars resulted in spring lines lower cold tolerance. In the case of substitution genes between two winter cultivars Mironovskaya 808 and Bezostaya 1 resulted in lines with shorter vernalization requirements and high frost tolerance level and lines with a longer expression of both these traits. The very important to know that developmental genes for vernalization acted as a master switch regulating the duration of expression of low temperature genes i. e. in winter wheat these genes are expressed for a longer time and at a higher level than in spring wheat.

The breeding process in different locations in regard to latitude, altitude and rainfall has proven a most efficient way to introduce and select genes for photoperiod insensitivity. The photoperiod insensitive genes, *Ppd1* and *Ppd2*, mostly present in spring wheat, and along with the dwarfing genes, *Rht1* and *Rht2*, resulted in a new plant type, which was not only lodging tolerant but higher yielding with high biomass due to pleiotropic effects or close linkage (Hoogendoorn et al., 1988).and provided adaptation to most irrigated wheat-growing areas. The *Rht1* and *Rht2* alone give a higher stem of wheat (over the 90 cm). The combination of both dwarfing genes would give a shorter stem (approximately 70 to 80 cm). There are additional height differences due to other minor gene effects. The *Ppd1* and *Ppd2* genes have individual effects on flowering. The presence of only one of these genes characterized intermediate flowering genotypes. Both genes effects of these genes are influence to early maturity.

The breeding under abiotic stress can directed to analyze changes in seed traits affected the root system in the successive generation. Combined stress of drought and high temperature had a substantial influence on the photosynthesis and respiration of winter and spring wheat. The basic changes can also, investigate by changes in the anatomic structure of the caryopsis. It was found that the pericarp and seed coat layers of stressed grains were thicker and the cuticle is more suberised. There were also great anatomic changes in the embryonic part of the caryopsis. Cultivar differences in the analyzed traits were observed. Plants grown under stress conditions mainly showed decrease or increase ((N, P, Ca, K, Mg, Na, B, and Mn).

Both genetically improved cultivars for yield and better scientific farming cultural methods have contributed to the yield increases (Zecevic et al., 2010). The effect of each factor (genetic/agrotechnic) is difficult to quantify. Yield stability has increased substantially across environments largely due to the adoption of management-responsive, high-yielding, disease-resistant semi dwarf wheat cultivars throughout all over the world. Improved agronomic practices also played an important role in enhancing the stability of yields. The increasing production and yield influenced by water-supply, (irrigation) applied nitrogen fertilizer (Kovacevic et al., 2009, Knezevic et al., 2011). Nitrogen fertilizer use depending of soil fertility and economic power. In average between 20 and 40 kg ha<sup>-1</sup> in some region farmers apply about 60 kg ha<sup>-1</sup>. That is not enough for numerous arable locations. On the other hand the high

rates of nitrogen applications, as well as heavy manuring, may increase disease incidence. In vegetable crops, research suggests about a 50-50 split between genetic gain and gain attributed to management. “Green revolution” varieties have increased yields 2 to 3 fold (Miflin, 2000).

Efforts to control insect pests of cereal crops generally take a back seat to the more pressing problems of abiotic production constraints, such as heat, drought, low soil fertility, salinity, day-length and so on. Even when these constraints are met, insect pests are frequently considered secondary to other biotic stresses, such as disease. The symptoms of insect infestation may be masked by various abiotic and biotic factors, or the insect itself once discovered may be misidentified and incorrect treatments prescribed. In some cases, insect damage is mistaken for that caused by drought, heat, cold or disease.

### Conclusion

Genetic diversity is the root of biodiversity and therefore important for agriculture, which are under the pressure factors such as climate change, loss of natural habitats, environmental degradation and population growth. Adopting methods to save plant diversity has the double benefit of protecting biodiversity and ensuring food security for farmers. Our interest is in how to make use of natural resources for the benefit of biodiversity to manage without risks and improve crop species by breeding.

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**IMPACT OF PERENNIAL APPLICATION OF NPK FERTILIZERS ON SOIL  
PROPERTIES OF VERTISOL SOIL TYPE**

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**Abstract**

The study was conducted in conditions of two-fields experiment (wheat-maize), formed in the year 1978, at the Vertisol soil type in the vicinity of Kragujevac. Vertisol at the beginning of the study was characterized by acidic pH, low content of available phosphorus, medium content humus, and high content of available potassium. The aim of this study was to determine influence of NPK fertilizers after 33 years of continuous application, on the basic elements of fertility (pH, humus, total N, available P<sub>2</sub>O<sub>5</sub> and available K<sub>2</sub>O). Nitrogen, phosphorus and potassium have been applied in two doses of 9 combinations, as: nitrogen in quantities of 80 and 120 kg/ha (N<sub>80</sub> and N<sub>120</sub>), and phosphorus and potassium in quantities of 60 and 100 kg/ha (P<sub>60</sub>, P<sub>100</sub> and K<sub>60</sub>, K<sub>100</sub>). Analyzes of chemical properties have been done in the year 2010, by the standard methods. The results indicate that after 33 years of application of NPK, with the exception of pH, it has taken to the improvement of all measured parameters of fertility. The biggest changes have been recorded in the content of available phosphorus. The content of this nutrient has been significantly increased even in the conditions of acid soil reaction. Increase in the content of available P<sub>2</sub>O<sub>5</sub> has been noted in the combinations of which it has been applied both individually and in combination with nitrogen and potassium. Application of higher doses of fertilizers have significantly increased content of Mn i Zn.

**Key words:** Vertisol, perennial application of fertilizers, NPK, manganese, zinc

**Introduction**

Perennial experiments with the application of fertilizers are important sources of information for understanding the factors that affect fertility of soil (Zhao et al., 2010), and its sustainable production (Regmi et al., 2002; Camara et al., 2003). The advantage of long-term research compared to short-term, are primarily being reflected in the fact that by these experiments are being gained information about the sustainability of fertilization treatments throughout several seasons. Over time, crop yields as well as the direction and intensity of change in soil properties, are being reflected on the rationality of the use of certain types of fertilizers, and the applied quantities, which provides economic and environmental importance of such experiments.

It is known that inorganic fertilizers besides of improving crop yields, directly or indirectly cause changes in chemical, physical and biological properties of soil. Some studies suggest that perennial use of mineral fertilizers, is resulting in deterioration of soil quality. Thus, the use of phosphorous fertilizers can lead to accumulation of heavy metals (Molina et al., 2009) or radioactive elements, Uranium - U i.e. (Stojanovic et al., 2006) and Thorium - Th (Wetterlind et al., 2012), and the long use of nitrogen fertilizers often leads to a reduction of soil pH (Bolan et al., 1991; Khonje et al., 1989). There are many more representatives of the

opinion that the rational application of fertilizers contributes to increase crop yields and improve product quality, but also improves the fertility of soil. Therefore, the aim of the study was to determine the effect of long-term application of N, P and K fertilizers on the basic elements of fertility of degraded vertisol, with an expressed acid reaction, as well as the content of the two trace minerals: manganese and zinc.

### Material and methods

Investigations were carried out on two-field stationary field trial at the Center for Small Grains in Kragujevac. Since 1978 it have began trials of the effect of perennial application of N, P and K fertilizers on the vertisol soil type properties, and yields of wheat and maize grown in two-field crop rotation. The main properties of vertisol in the study were acidic pH and very low phosphorus content.

Individual N, P and K fertilizers were applied in two quantities and nitrogen at a level of 80 and 120 kg N ha<sup>-1</sup> (variant N<sub>80</sub> - V2 and N<sub>120</sub> - V3), phosphorus at 60 and 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (variant P<sub>60</sub> - V4 and P<sub>100</sub>- V5) and potassium salt at 60 and 100 kg K<sub>2</sub>O ha<sup>-1</sup> (variant K<sub>60</sub> - V6 and K<sub>100</sub> - V7). Nutrients were applied in the two mutual combination, or in quantities of 80 kg N ha<sup>-1</sup> and 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 60 kg K<sub>2</sub>O ha<sup>-1</sup> (variant N<sub>80</sub> P<sub>60</sub> K<sub>60</sub> - V8) in quantities 120 kg N ha<sup>-1</sup>, 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 60 kg K<sub>2</sub>O ha<sup>-1</sup> (variant N<sub>120</sub>P<sub>100</sub>K<sub>60</sub> - V9). Treatments have been compared with control where fertilizers have not been applied, or with a variant that has not been fertilized (V1). In total there have been 9 combinations which have been repeated 4 times, and the design of the trial was a random block design (ANOVA).

There have been used single nitrogen a (CAN or urea), single phosphorus (superphosphate) and single potassium (40% K salt) fertilizers. Applied technology of cultivation, planting and care of crops has been done by the standards for breeding of wheat and maize.

Average soil samples for analysis have been taken from the surface up to of 20 cm depth in the autumn of 2010. Formation of average samples has been done at level of basic plot and the samples have been represented by a combination of the given plots.

Soil pH has been measured with pH-meter with a glass electrode in 1:2.5 suspensions with distilled water (active acidity) and 1N KCl (substitution acidity). Available P and K have been determined by the method of AL Egner-Riehm. The examined elements have been first extracted in aceto-lactate solution, afterwards potassium content has been read on the from the filtrate at flame photometer, and phosphorus content has been determined in a spectrophotometer after coloring filtrate by ammonium molybdate and SnCl<sub>2</sub>. Humus has been determined by the Kotzmann's method and total N using the Kjeldahl method. Available manganese and zinc have been determined by atomic absorption spectrophotometry (AAS) after extraction of Mn in 0.05 M H<sub>2</sub>SO<sub>4</sub> and Zn in 1 M CH<sub>3</sub>COONH<sub>4</sub> (pH = 4.8).

Data have been analyzed using standard statistical methods of analysis of variance (ANOVA) using Microsoft Excel 2007 and Statistical Program 5.0. Data analysis has been used to interpret the results and draw conclusions.

### Results and discussion

Application of N, P and K fertilizers in the period of 33 years has contributed to significant changes in some parameters of vertisols fertility, the direction and intensity of the changes, compared to unfertilized treatments, has been depended on the type and quantity of fertilizers that have been entered, and the results are given in Table 1. The measured pH values indicate that significant changes of vertisol soil type occurred after years of applications of all types of fertilizers. The biggest changes occurred in the treatment where there has been applied only N and slightly lower in the variant of applied P, and their

fertilizers compared to control increased substitution acidity of vertisol, more than 0.20 pH units, which is a very significant difference ( $p < 0,01$ ). The influence of nitrogen fertilizers on soil acidification and its further destruction is well known and confirmed by many perennial experiments (Barak et al., 1997; Zhao et al., 2010). On the other hand, NPK variants compared to the control contributed to a slight reduction of pH (V9), or led to a slight increase.

Other elements of fertility have been improved and in accordance with the type of applied fertilizer. The content of humus and total N in all variants of fertilization increased compared to unfertilized. Although the importance of perennial nitrogen fertilization is often being emphasized on maintaining or increasing the content of organic matter (Bundy et al., 2011) these studies have shown a better effect of individual P and K, NPK fertilizers and it can be said that it has largely contributed to an increase of organic carbon and total N content (Tong et al., 2009) and nitrate nitrogen (Zhang, 2012)

Table 1. Characteristics of clay soil fertility after 33 years of fertilization

Variants	pH		Humus (%)	N (%)	mg · 100 g <sup>-1</sup>	
	H <sub>2</sub> O	KCl			P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
V1	5.54	4.28	2.417	0.165	4.50	22.43
V2	5.32	4.03	2.840	0.178	3.88	21.33
V3	5.08	4.06	2.995	0.173	5.33	25.65
V4	5.36	4.12	3.055	0.170	13.38	25.18
V5	5.14	4.12	3.270	0.175	13.63	21.75
V6	5.42	4.20	3.133	0.165	4.25	30.68
V7	5.29	4.16	3.108	0.178	3.50	33.80
V8	5.11	4.34	3.243	0.178	15.38	31.63
V9	5.28	4.19	3.023	0.185	25.50	35.63
Lsd 0.05	0.13	0.09	0.304	0.016	5.04	3.03
Lsd 0.01	0.17	0.12	0.410	0.021	6.81	4.09

Considering an available phosphorus and potassium in vertisol it has been determined that the fertilizers containing these elements, individually or NPK, had direct impact on their concentration in the soil. After 33 years in the most physiologically active P<sub>2</sub>O<sub>5</sub> content has been recorded in NPK variants where phosphorus is added in a quantity of 100 kg ha<sup>-1</sup> (V9), followed by NPK variant with 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (V8). Individual P-fertilizers have also contributed to the increase of phosphorus content and the differences between these variants and where fertilization has not been applied are highly significant. Thus, perennial application of fertilizers with phosphorus (individual and NPK) has been strongly influenced on its content in the soil, contributing to the accumulation of available forms in the area of application (Richards et al., 1998; Morocco et al., 1999, Otto and Kilian, 2001; Cakmak et al., 2010; Selles et al., 2011), and on this balance of available phosphorus certainly influenced his incomplete use. A similar trend was recorded for the effect of fertilizers on the content of available K<sub>2</sub>O. The only difference is that there is less visible effect of NPK fertilizer on potassium content increased, compared to the variants where it have been applied only K-fertilizers.

The content of available Mn in the vertisol have been within the medium content and in all the variants of fertilization it has been significantly increased compared to the control (Figure 1). The difference appeared among the same fertilizer treatments but with different amounts of active ingredients. On that occasion, all the variants where it has been applied

large quantities of active substances has been increasing Mn content. The content of available Mn differences among variants of the same fertilizer but with different amounts of active ingredient were not statistically significant. However, higher values of Mn at variant with applied higher doses N (V3), P (V5) and K (V7) compared to the control were highly significant ( $p < 0.01$ ). The exception is  $N_{120}P_{100}K_{60}$  version (V9).

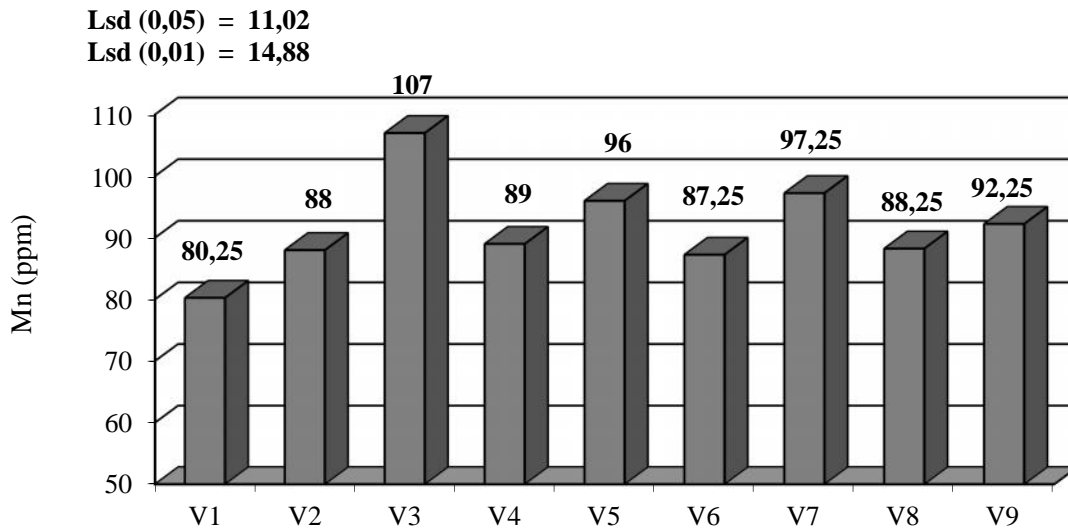


Figure 1. Impact of years of fertilization on the content of available Mn (ppm)

The trend of higher available manganese content with increasing amounts of fertilizer it has been observed at a concentration of available Zn in vertisol, and differences were very significant compared to the control and in relation to a combination fertilizer with a lower intake of active ingredient (Figure 2).

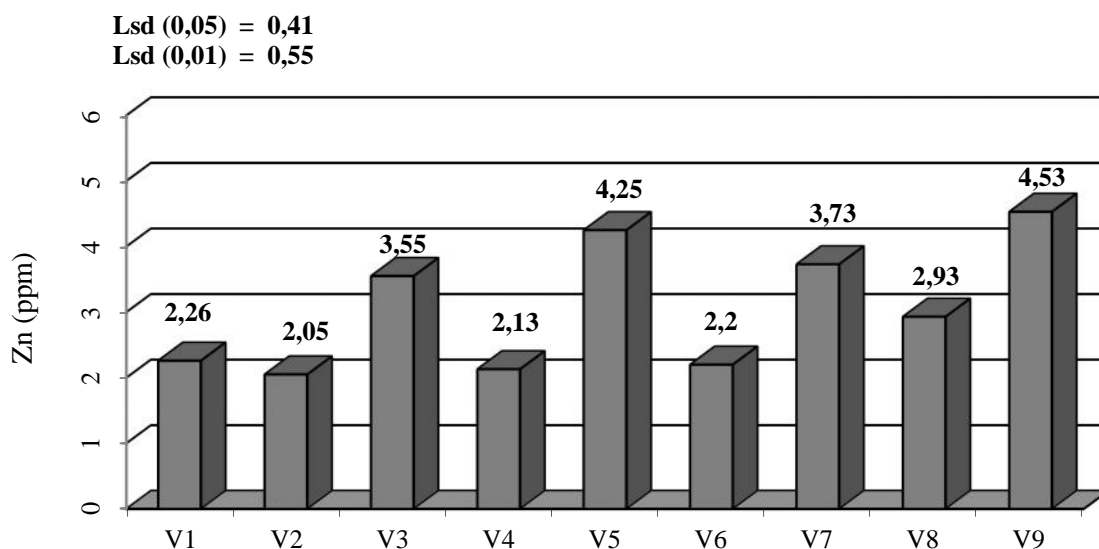


Figure 2. Influence of perennial fertilizing on the available Zn (ppm) content

It should be emphasized that in variant were applied lower doses of N (V2), P (V4) and V (6), there was a decrease of the content of available Zn compared to the control. The exception is the NPK variant (V8) where it has been noted a slight increase in Zn content. Experiences with the impact of fertilizers on the content of available forms of trace elements, including Mn and Zn in the soil are different. Mainly they deny an important role of fertilizers on change of concentration (Rutkowska et al., 2009) by highlighting the growing importance of perennial application of organic fertilizers versus to mineral (Li et al., 2010; Richards et al., 2011). On the other hand there is the view that the content of trace elements, in addition to organic, can be affected by mineral fertilizers (Thakur et al., 2011), especially phosphorus (Molina et al., 2009) which contain heavy metals (As, Cd, Cr), and numerous micronutrients, especially Zn and P fertilizer application may result in the increase of their concentration in the soil, which should be taken into account.

### Conclusion

Continuous application of fertilizers has affected the change of vertisols fertility parameters. Nitrogen fertilizers increase the substitution acidity and contributed to the acid destruction and further degradation of the soil type. Phosphorus and potassium were significantly increased only in the variant with the application of these two elements, and humus and total nitrogen for all the fertilization variants. Fertilization, especially the application of larger amounts of fertilizers, contributed to the increase of the concentration of available Mn. Higher doses of fertilizers significantly influenced the increase of Zn content, while the intake of small amounts of fertilizer decreased the content of this element. After 33 years of fertilization, the achieved level of available  $P_2O_5$ ,  $K_2O$ , Mn and Zn, make the caution, because over the time concentration of these elements can increase to the undesirable economic and environmental level.

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## **THE IMPACT OF DIFFERENT TECHNOLOGIES OF PRODUCTION OF RED WINES ON THE ORGANOLEPTIC SCORE IN THE POPULATION OF YOUNG WINEMAKERS**

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### **Abstract**

The value of wine is determined by a number of parameters, and besides physical - chemical and organoleptic parameters, which are influenced by the quality of grapes and wine, important role has tradition, marketing and consumer trends in wine. The aim is to show the trends and preferences of future wine experts who will form the styles of wine, and who are themselves educated wine consumers. The experiment was conducted in a teaching facility of University of applied sciences in Požega. In a study students from the second and third year of professional study Vineyards - Wine – Fruit growing participated. Students were randomly selected and divided into three commissions of five members. Assessed the wine Pinot Noir, Merlot, Cabernet Sauvignon and Syrah. Harvest of each cultivar was carried out on the same day so that the parameters of grapes for each wine the same whether it is a wine produced in a stainless steel bowl or barriques. Wines produced exclusively in stainless steel containers retain more varietal characteristics and fruitiness and are fresher than the wine from barrique barrels. All samples of wine vintages are 2012<sup>th</sup>. Sensory evaluation of wines was carried out using 100 positive points (OIV). All results were statistically analyzed. Wines in barriques with Pinot Noir, Merlot and Cabernet Sauvignon achieved better grades.

**Keywords:** Barrique, organoleptic, red wine, young

### **Introduction**

One of the ideas for this research comes from the title of the book: “the Paris judgment, California against France and historic Paris tasting in 1976” that has revolutionized the world of wine (Taber, 2009). The book shows how great the impact of tradition, fashion and trends are in forming attitudes of producers and consumers of wine.

The value of wine are determined by a number of parameters, and besides physical - chemical and organoleptic parameters, which are influenced by the quality of grapes and wine, important role has tradition, marketing and consumer trends in wine (Jackson, 2000). The aim is to show the trends and preferences of future wine experts who will form the styles of wine, and who are themselves educated wine consumers.

Comparison of red wine produced exclusively in stainless steel canisters and barriques were conducted because it was observed that most tasters lately prefer fruit, fresher wines.

In support of this style of wine goes and production of wine in barriques that raises the price of wine.

### **Materials and methods**

In a study students from the second and third year of professional study Vineyards - Wine – Fruit growing participated. All students are trained in sensory evaluation of wine. Students were randomly selected and divided into three commissions of five members. Each committee represents one repetition of experiments which is set in completely randomized Pinot Noir, Merlot, Cabernet Sauvignon and Syrah were assessed. Harvest of each cultivar was carried



out on the same day so that the parameters of grapes for each wine are the same whether it is a wine produced in a stainless steel bowl or barriques. Basic parameters of tasted wines are shown in Table 1, and all the samples belong to the category of dry wine.

Table 1. Samples of wines that have been used in research

Wine	Alcohol (vol %)	Total acidity as tartaric (g/l)
Pinot Noir - stainless steel	17	6,5
Pinot Noir - Barrique LT	16,3	5,5
Merlot - stainless steel	16,1	5,2
Merlot - Barrique LT	15,7	5,5
Cabernet Sauvignon - stainless steel	14,5	5
Cabernet Sauvignon - MT	13,7	5
Syrah - stainless steel	15	5,2
Syrah – barrique - HAT	16	5,7

All samples of wine are vintage 2012th, the wines that were in barriques were selected in the previous tasting in order to better highlight the characteristics of a positive impact of barrique barrels. Sensory evaluation of wines was carried out using 100 positive points (OIV), (NN106/04, 2004). After the results were separated by an average score of smell, taste and harmony, and overall rating of wine. Color and purity are not especially prominent because it is a young red wines and all the wines in parameters of color and purity assessed maximum rating. The results were analyzed in statistically variation.

### Results and discussion

Tables 2, 3, 4 and 5 presents the average values of smell, taste, harmony and overall rate of wines that are produced in stainless steel containers and in barriques. Wines produced exclusively in stainless steel containers retain more varietal characteristics and fruitiness and are fresher than the wine from barrique barrels.

Table 2. Smell of wine varieties Pinot Noir, Merlot, Cabernet Sauvignon and Syrah, made in stainless steel canisters and barriques, vintage 2012th

	Pinot noir	Merlot	Cabernet Sauv.	Syrah
Stainless steel	24,7	23,2	24,1	23,3
Barrique	26,1	26	27	24,8
Significance	n.s.	n.s.	*	n.s.

\*\*, \*, n.s. significant with  $p = 0,01, 0,05$  or insignificant to LSD test

Table 2 shows that evaluators are better assessed the odors of wines produced in barriques, although statistically significant better smell only wine Cabernet Sauvignon.

Table 3. Taste wine varieties Pinot Noir, Merlot, Cabernet Sauvignon and Syrah, in stainless steel canisters and barriques, vintage 2012th

	Pinot noir	Merlot	Cabernet Sauv.	Syrah
Stainless steel	36,7	33	33,6	35,7
Barrique	37,6	37	37,8	34,5
Significance	n.s.	n.s.	**	n.s.

\*\*,\* ,n.s. significant with  $p = 0,01, 0,05$  or insignificant to LSD test

The biggest impact on sensory perception is tastes, probably more important than smell and affects the overall assessment to food in general and wine (Clark & Baker 2004). Commission significantly better evaluate flavor varieties Cabernet Sauvignon produced in barriques compared to wine from stainless steel containers. Pinot Noir and Merlot from wooden barrels also have left a better impression on the tasters. It is only a taste of the variety Syrah from stainless steel containers better assessed in relation to wine produced in wood barrels, but the difference in the number of points is very small and not statistically significant. The fact that the evaluators better recognize and appreciate the taste exactly the Cabernet Sauvignon probably partly lies in the varietal characteristics of the variety, which is expressed with almost aggressive flavors with notes of black currant and herbs, which are especially pronounced in the young wine, considering that it is a wine harvest 2012th year. It is in this wine tasters are the easiest to recognize the positive impact of barrique barrels of wine.

Table 4. Harmony and the general impression of wine varieties Pinot Noir, Merlot, Cabernet Sauvignon and Syrah, made in stainless steel canisters and barriques, vintage

	Pinot noir	Merlot	Cabernet Sauv.	Syrah
Stainless steel	10	9,4	9,2	9,4
Barrique	10,2	9,8	9	9,7
Significance	n.s.	n.s.	n.s.	n.s.

\*\*,\* ,n.s. significant with  $p = 0,01, 0,05$  or insignificant to LSD test

Table 4 shows that all the Commission assess an equally characteristic harmonies and the general perception of wine from stainless steel and barrique barrels and no significant differences in the preferences of tasters. The reason for this result probably lies in the fact that the range of passing to score great all three points.

Table 5. Total Score wine varieties Pinot Noir, Merlot, Cabernet Sauvignon and Syrah, made in stainless steel canisters and barriques, vintage

	Pinot noir	Merlot	Cabernet Sauv.	Syrah
Stainless steel	87,5	80,7	81,9	83,4
Barrique	88,9	87,8	88,8	83,9
Significance	n.s.	n.s.	**	n.s.

\*\*,\* ,n.s. significant with  $p = 0,01, 0,05$  or insignificant to LSD test

On the basis of the total score is evident that tasters better rated wines of all varieties produced in barriques. Wine Cabernet Sauvignon from wooden barrels rated significantly better than the wine in stainless steel. With Pinot Noir and Syrah differences are very small. Both Pinot Noir wine tasters have rated as a top-quality wine, while the Syrah categorized as quality wine. Merlot and Cabernet Sauvignon from stainless steel containers have been

categorized as a quality wine, while their version of barriques rated as a top-quality wines. From the results it is evident that young educated tasters prefer red wines produced in wooden barrels, although all the results are not statistically significant.

### **Conclusion**

Young educated tasters and future wine producers better rated wines of all varieties produced in barriques compared to wines produced in stainless steel containers. Statistically significant better rated wine varieties are Cabernet Sauvignon produced in barriques compared with the same wine in stainless steel containers. Only the taste of wine Syrah from stainless steel containers better assessed in relation to parallel wine from wooden barrels but the difference in scores was not statistically significant. It would be good to expand the study to a larger sample that is to increase the number of commissions (repetition) that the results would be as representative as possible. In the end, you should always keep in mind that the rate of experienced wine tasters is partly a result of their subjective perception (Domine, 2004).

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**MORPHOGENETIC FEATURES OF UNIVERSITY  
LEGUMES COLLECTION**

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**Abstract**

This study has been conducted to highlight the outputs of 2011-12 crop rotation under mountain and steppe (plain) zones in Almaty Region. Principal morphogenetic properties have been studied across the collection of common bean, *Phaseolus vulgaris* L. (Kazakhstani, American, Chinese, Czech, Polish, Russian, and Turkish collections) from different soil and climate areas under mountain and steppe zone conditions of Almaty Region. A number of useful genetic stocks for major economically valuable traits has been identified.

Stock varietal resources have been studied on morphogenetic features. It has been shown that cv. “Luna” from Czech collection would be the earliest by ripening (80 days from the onset of ontogenesis to complete technical ripeness). Other varieties could reach the same state 10-12 days later. Using local “Aktatti” line the effect of new domestic bioorganomineral fertilizer has been shown on morphogenetic traits of common bean plants.

Basic catalogue of stock common bean resources including nearly 40 parental common bean and related cultivars of diverse geographic origin has been compiled to be completed this year by 6 French cultivars of bush and liana common beans (Argus, Coco nain blanc precoce, Triomphe de Farcy, Merveille de Venise, Mistica, and Phenomene manufactured by Truffaut and Vilmorin Ltd.). Investigation on growing domestic collection of cultivars and lines is also in progress.

**Keywords:** common bean, catalogue of stock resources

**Introduction**

Biological features of common bean

The process of cultivar- as population or hybrid generation for common bean, *Phaseolus vulgaris* L. may be predicted from natural hybridization and natural selection of those forms which are adapted to changing climate conditions within the borders of their areals, and as a consequence, crop's spreading out of those areals to strange territories. Transformation of wild species became especially extensive with human participation, when most appropriate forms from points of feeding, life and cropping conditions had been set out to be sought for (Bodnar & Lavrinenko, 1977).

In the course of evolution bushy, large-leafy, early-ripening forms with determinant type of growth, large and average flowers, with non-dehiscent and lacking pergament layer pods (Zhukovsky, 1971)

Comparing to other legumes, common bean occurred to be more capricious to the soil preferring fertilized sand soils or light clay-containing soils. Cold clay soils moisture by the underground waters are considered to be inappropriate for common bean. Soil swamping caused by high acidification may decrease crop harvests, too (Popov & Martynov, 2001). Due to acidic soils the development of nitrogen-fixing (tuber) bacteria is detained, and the effect

of the nitragine is weakened (Zerfus et al., 1997). Neutralization or lime formation (calcification) of acidic soils lead to increasing yields of common bean.

Under mountain and steppe (plain) conditions of Almaty Region morphogenetic traits for 37 collection cultivars of common bean from different soil and climate zones (Kazakhstan, Chinese, Polish, Russian, Turk, and Czech collections) have been assessed.

### **Materials and methods**

This study has been fulfilled under 2011-2012 field crop rotation in mountain and steppe (plain) zones of Almaty Region. 37 specimens of common bean and its relations have been sown for: i, generation and study on domestic cultivars of common bean; ii, setting up the collection to be processed together with the students; iii, development of field and seed research at new “Zhanga Talap” Agrobiocenter of al-Farabi Kazakh National University. Research material has been partly registered as the State Certificate on the subject of author rights No. 612 of 14 May, 2012 titled: “Distribution and exchange of the specimens of grain legume crops”.

Investigation on the collection items has been performed according to a Vavilov Institute and Awassa Agricultural Research Center prescriptions (Korsakov, 1975; Asfaw et al., 2009). Samples have been sown on plots of 2 x 10 square meters. Double-row sowing with wide inter-row spacing (40-60 cm) has been applied. Seeds have been collected without mechanic engines. Form “Aktatti” has been used as a standard distributed in Almaty Region. Seed collection has been sown not less than in two replicas. Observations, measurements and assays have been conducted in accordance with “Methodical Instructions on The Study of The Collection for Grain Legume Crops” issued by a Vavilov Institute and relying on the Classifier’s gradations for the genus *Phaseolus* L. (Budanova & Lagutina, 1979). To provide computer planning of the specimens to be planted, the own software programme entitled “Planting manager” (the State Certificate on the Subject of Authorship Rights No. 1034 of 1 August, 2012) has been implied.

Structural analysis of common bean plants has been carried out by a range of main traits such as: stem height in cm; length and width of upper leaf at 30-th, 45-th and 65-th days of vegetation, cm; number of pods and number of seeds per plant in pieces; number of seeds per pod; weight of seeds per plant (seed productivity) in g; 100-seed weight, g, and others. Average leaf elongation or width extension has been calculated in percentage (%) as average value between the upper leaf length at 30-th day and 45-th day of vegetation compared with the same parameter for the 65-th day of vegetation.

Statistic treatment of the data obtained has been performed by the technique of variation statistics (Dospekhov, 1985; Bisgaard, 2008).

### **Results and Discussion**

Seed stocks for the mountain zone have been sown at two sites, and namely: i, the territory of the Institute of Botany and Phytointroduction of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan; ii, mountain plot in the Almarasan Gorge along the River Bolshaya Almatinka (800-1200 m above the sea level). Seed stocks for the plain zone have been sown at the territory of “Zhanga Talap” Agrobiocentre of al-Farabi Kazakh National University and Kazakh Institute of Soil Science and Crop Research (both nearly 600 m above the sea level).

17 cultivars and lines of common bean, *Phaseolus vulgaris* L. have been planted in the mountain zone (9 - at the territory of the Institute of Botany and Phytointroduction ; 8 - mountain plot in the Almarasan Gorge). 20 cultivars and lines of common bean and its

relations (broad bean, *Vicia faba* L. and Turkish beans, *Phaseolus coccineus* L.) have been planted in the steppe zone (“Zhanga Talap” Agrobiocenter). With reference of the Czech collection of introduceable cultivars, it has been established that at the 30-th day after the sowing cv. “Zuzka” has displayed the highest germination rate (53.0%). Two other Czech cultivars have revealed germination degrees of 23.3% (cv. “Katka”) and 16.6% (cv. “Luna”). Cv. “Zuzka” has also been determined to surpass other cultivars by leaf size (11.2 x 8.0 cm), whereas these parameters for cvs. “Katka” and “Luna” have made up 6.5 x 4.5 and 9.3 x 6.4 cm, respectively. At the same time it has been noticed too that cv. “Zuzka” would be characterized by earlier transition to the stage of flower formation.

Morphogenetic studies of stock genetic resources for breeding and phenological observations over the process seed germination. Using patterns of Czech and domestic cultivars and lines, it has been indicated that local lines would take over Czech samples to be introduced by the rate of germination (see Table 1).

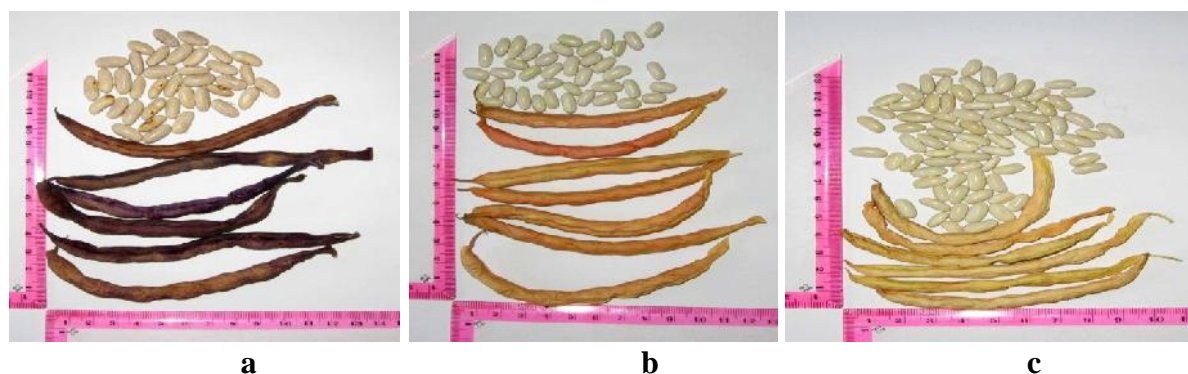
Germination values for cv. “Zuzka” have reliably exceeded similar data for other Czech cultivars under the study.

It has been shown that common bean leaves under local conditions have possessed egg-like or transitional to wide egg-like form. Some cultivars and lines have been recorded to have silver-polished stipules and differences in leaf colour which is known to be a characteristic genetic trait intrinsic for the cultivar. This trait is dependent on plant age, the extent of soil fertility and fertilizer concentrations which were induced.

Table 1. Results of phenological observations: intermediate evaluation of germination rates for Kazakhstan and Czech specimens of common bean (30-th day after sowing)

No	Cultivar or line	Germination, %
1	Zuzka	53.33± 0.15***
2	Katka	23.33± 0.10***
3	Luna	16.67± 0.10
4	Nazym	78.26± 0.25***
5	Talgat	50.00± 0.20***
Footnote: *** <0.001		

Propagation of common bean collection in Kazakhstan is in progress. One of the factors for positive dynamics is a great polymorphism of stocks under ongoing study and possibility to introducing foreign samples, and in that particular case Czech collection. In the mountain zone of Almaty Region (mountain plot in the Almarasan Gorge) it has been established that three cultivars, cvs. “Zuzka”, “Katka”, and “Luna” (Fig. 1) from four varieties of Czech collection attempted to be introduced in 2012 would indicate traits of high productivity with the exception of cv. “Jitka” which didn’t sprout at all.



**Fig. 1.** Czech cultivars at the stage of technical ripeness. **a**, cv. “Zuzka”; **b**, cv. “Katka”; and **c**, cv. “Luna”.

Seed material obtained for cvs. “Zuzka”, “Katka”, and “Luna” would be used for further propagation in the steppe zone, at the territory of “Zhanga Talap” Agrobiocentre. However, all Czech cultivars have shown high susceptibility to bean weevil (*Acanthoscelides obtectus* Sav.) at room temperature. The most early ripening cultivar has been detected to be cv. “Luna” that had a maturation period of 80 days from the very onset of ontogenesis. Other cultivars have ripen to reach the stage of technical ripeness 10-12 days later.

As seen from table 2, maximal size of ripen pod on the 92-th day since planting is typical of cv. “Zuzka” ( $13.3 \pm 0.1$  cm). The same value for cvs. “Katka” and “Luna” has been determined to make up  $12.0 \pm 0.2$  cm and  $10.8 \pm 0.1$  cm, respectively. Local line “Aktatti” has close pod length in range of  $11.0 \pm 0.1$  cm (the data are not presented in Table 2), whereas other local lines, “Nazym” and “Talgat” have exhibited in the same period mature pod length reaching  $12.4 \pm 0.1$  and  $9.0 \pm 0.2$  cm, respectively.

Pod length values for cv. “Zuzka” and line “Talgat” have reliably excelled the same parameter of other cultivars and lines under investigation.

In the steppe zone (“Zhanga Talap” Agrobiocenter) patterns of American, Polish and Russian common bean have been successfully propagated. It has been demonstrated that cvs. “Bijchanka”, “Camelia”, “Red Goya” and “Ufimskaya” introduced would be most adapted to the steppe zone.

Using local “Aktatti” line the effect of new domestic bioorganomineral fertilizer has been shown on morphogenetic traits of common bean plants.

Table 2. Outputs of structural analysis of Kazakhstan and Czech cultivars and lines of common bean

No	Cultivar or line	Number of seeds per pod (92-th day of vegetation)	Pod length (92-th day of vegetation)
1	Zuzka	$6 \pm 1$	$13.3 \pm 0.1^{***}$
2	Katka	$7 \pm 1^*$	$12.0 \pm 0.2^{***}$
3	Luna	$13 \pm 1^{***}$	$10.8 \pm 0.1^{***}$
4	Nazym	$4 \pm 1$	$12.4 \pm 0.1^{***}$
5	Talgat	$4 \pm 1$	$9.0 \pm 0.2$

Footnote: \* <0.05; \*\*\* <0.001

### Conclusions

Based on the survey over morphogenetic traits of available seed stocks, the catalogue of main parental cultivars for common bean has been designed. It has included nearly 40 parental specimens of common bean and its relations of diverse geographic origin. These cultivars and lines are supposed to be completed this year by 6 French cultivars of bush and liana common beans (“Argus”, “Coco nain blanc precoce”, “Triomphe de Farcy”, “Merveille de Venise”, “Mistica”, and “Phenomene” manufactured by Truffaut and Vilmorin companies). Investigation on growing domestic collection of cultivars and lines is also in progress to be studied by biochemical, cytogenetic and other properties as used for breeding purposes.

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## EXAMINATION OF SOME DUTCH RED SKIN POTATO VARIETIES IN DIFFERENT AGRO-ECOLOGICAL CONDITIONS OF MONTENEGRO

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### Summary

The paper presents results of productivity research of six potato red skin varieties in three different locations in the mountainous regions of Montenegro: Nikši (800 meters of altitude), Kolašin (900 meters of altitude) and Žabljak (1450 meters of altitude). The study took place during 2010 and 2011, and following varieties were tested: Kondor, Kuroda, Aladin, Roko, Desiree and Rudolph.

The highest tuber yield in two-year average had variety Rudolph (32.8 t.ha<sup>-1</sup>), while the lowest yield was measured in the crop of Kuroda variety (25.4 t.ha<sup>-1</sup>).

As the agro-ecological conditions in the studied area varied, the varieties reaction differentiated as well. Highest productivity was in Žabljak (29.1 t.ha<sup>-1</sup>), while the lowest was in Nikši (28 t.ha<sup>-1</sup>).

Higher potato yields were obtained in 2010 (28.9 t.ha<sup>-1</sup>) as the result of the higher total amount of rainfall during the potato vegetation period and slightly better monthly distribution. Nevertheless, interactions location x year, variety x year and variety x location x year resulted in statistically significant difference in yield.

**Key words:** red skin potato varieties, agro-ecological conditions, productivity

### Introduction

Potato (*Solanum tuberosum* L.) is the third most important crop in human diet after rice and wheat (FAOSTAT, 2008; CIP, 2011). According to the data of Food and Agriculture Organization of the United Nations (FAO) from 2008, potato was the most important non-cereal crop in human diet.

So far in the world have been selected few thousands of potato varieties among which dominate varieties with yellow skin color. Although the popularity of red skin color varieties increased lately, the surfaces planted with these varieties are still relatively small. Potato varieties with red skin color today are mostly grown in the Mediterranean area, Middle East and North Africa. These varieties, regardless of the flesh color, have high nutritional value and are generally used for direct consumption. Due to the firmness and texture of flesh they are commonly used for soups, salads and frying. Although the skin and flesh color does not affect the quality of the tubers, it significantly determines customer interests. Stability of flesh color after cooking significantly affects its marketability (Momirović et al., 2000).

Natural pigmentation of the skin color makes a group of varieties with red skin color very attractive for supermarkets and green markets (McComber et al., 1994). These varieties are characterized by a slightly higher content of fiber, vitamin C, iron and protein. Although the market is relatively small and unstable for red skin varieties noticeable increase in the consumption of red varieties in the human diet is a consequence of a high content of anti cancer substances in the tubers - anthocyanins and various antioxidant substances (Brown, 2005; Wang and Stoner, 2008).

The study of the different potato varieties on a numerous localities is very important as it helps making the best selection of genotypes adapted to the given agro-ecological conditions (Yang, 2002, Jovovi et al., 2012a, 2012b). Some potato genotypes have the ability to adapt to very different environmental conditions and in the long run provide stable yields to a higher or lower level (Annicchiarico, 2002). Only through knowledge of biological, morphological and productive characteristics of potato varieties, and their reactions to different agro-ecological conditions it is possible to achieve high and stable yields (Momirovi et al., 2000). Productivity is a function of the variety of its adaptability to environmental conditions, so it is very important to choose varieties that will be able to consistently achieve high yields in a wide range of different environmental factors (Haldavankar et al., 2009).

The aim of this study was to examine the genetic potential of six dominant red varieties of potatoes in the conditions of the mountain region of Montenegro in order to find the genotypes that will obtain stable and satisfactory yields in these conditions. In order to ensure high and stable production, which involves the maximum use of the genetic potential, it is necessary to have information on different types of interactions between genotype and the environment (Jovovi et al., 2013c).

#### Materials and methods

The study of the productivity of six Dutch potato varieties with red skin color, predominantly grown in Montenegro (Kondor, Kuroda, Aladdin, Roko, Desiree and Rudolph) was conducted during 2010 and 2011. The experiments were conducted in different climatic conditions and different soil types: Nikši (800 meters above sea level, acid brown soil), Kolašin (900 meters above sea level, alluvial-diluvial soil) and Žabljak (1450 meters above sea level, black soil on limestone).

Tests were carried out using field trials in a randomized block design with 4 replications. The plot size was 21 m<sup>2</sup>. Planting of potatoes was done manually with 70 cm between row distance and 33 cm within row plant distance respectively, achieving the density of 43300 plants per hectare. Standard agricultural practice for the potato crop was applied. Potato harvesting was done after full maturation of canopy. The potato yield in the experiment was determined by measuring the tubers at each elementary plot, and then the yield per hectare was calculated.

The analysis of variance was calculated according to randomize complete block design with three factors: variety (V), locality (L) and year (Y). The significant differences among the means were evaluated according to least significant difference (Lsd) test (Maleti, 2005).

Soil on which the experiments were carried out is characterized by favorable water and air properties and high humus content. On the other hand these soils are poor in phosphorus and calcium and with moderate potassium content (Table 1).

Table 1 - Chemical characteristics soil on experimental field

Depth (cm)	Locality	pH		CaCO <sub>3</sub> %	Humus %	Soluble mg/100 g	
		H <sub>2</sub> O	nKCl			P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
40	Nikši	6.57	5.97	1.75	3.05	9.3	17.3
	Kolašin	6.29	5.55	1.1	4.53	2.4	16.0
	Žabljak	5.91	4.85	2.05	7.32	6.2	23.5

As shown in Table 2 meteorological data were significantly different on different sites and between years as well.

Table . 2 - Meteorological conditions during the experiments

Year	Locality	Month					Average
		May	June	July	August	September	
Air temperature ( $^{\circ}\text{C}$ )							
2010	Nikšići	14	18.6	22	23.3	16.1	18.8
	Kolašin	11.6	15.9	18	18.3	12.6	15.3
	Žabljak	9.2	14.1	16.0	17.1	10.7	13.4
2011	Nikšići	15.6	18.5	21.8	22.8	18.1	19.4
	Kolašin	11.3	16.2	17.6	18	15.6	15.7
	Žabljak	8.8	13.9	15.9	16.5	14.1	13.8
Amount of rainfall (mm)							
2010	Nikšići	157	220	39	23	210	649
	Kolašin	202	103	34	12	117	468
	Žabljak	163	93	35	20	82	393
2011	Nikšići	135	56	40	29	140	400
	Kolašin	148	72	70	40	101	431
	Žabljak	162	43	76	53	113	447

## Results and discussion

The average potato yield in investigation years is shown in Table 3. Highest potato yield in two years study was obtained in variety Rudolph -  $32.8 \text{ t}\cdot\text{ha}^{-1}$ , and lowest in Kuroda -  $25.4 \text{ t}\cdot\text{ha}^{-1}$ . High potato yields were also measured in varieties Aladin and Roko ( $29.6$  and  $29 \text{ t}\cdot\text{ha}^{-1}$ , respectively). Differences in yields obtained between variety Rudolph and other varieties were statistically very significant. Also, all varieties gave significantly higher tuber yield compared to Kuroda. Significant differences in the genetic bases among the studied potato varieties is in compliance with the results cited by Jovović et al. (2012d), Broić et al. (2000) and Bugarić et al. (1997).

Results of research demonstrated significant impact of the localities on potato yield of studied varieties. Highest yields were obtained in Žabljak ( $31.3 \text{ t}\cdot\text{ha}^{-1}$ ) in 2011, and lowest in Nikšići ( $25.3 \text{ t}\cdot\text{ha}^{-1}$ ) in the same year. According to location means, the highest potato yield for all varieties was achieved in Žabljak ( $29.1 \text{ t}\cdot\text{ha}^{-1}$ ), while the lowest in Nikšići ( $28 \text{ t}\cdot\text{ha}^{-1}$ ). Statistical processing of data significant differences was traced exclusively in average yields of potato tubers in localities Žabljak and Nikšići. This strong influence of environment on yield of potato has also been reported by Hassanpanah (2011).

By analysis of average potato yields, it can be concluded that in studied years, yields were more or less uniform -  $28.9$  in 2010 and  $28.2 \text{ t}\cdot\text{ha}^{-1}$  in 2011. Taking into account that the potato plants require temperatures between  $16$  do  $19 \text{ }^{\circ}\text{C}$  in phase of tuberisation, intensive tuber growth and beginning of flowering (Barkley, 2005) it is obvious that the potato crop on all localities in both studied years had favorable thermal conditions (Table 2). Higher precipitation and slightly better monthly distribution caused the slight increase of yield in 2010. The difference in tuber yield in studied years had 5% significance level. Since the research was conducted in the northern region of Montenegro, in the mountain climate conditions, the tuber yield obtained can be considered high.

Table 3 - Potato yield in investigated period

Variety (A)	Locality (B)									Average (A)
	Nikši			Kolašin			Žabljak			
	2010	2011	Average	2010	2011	Average	2010	2011	Average	
Kondor	29.7	25.5	<b>27.6</b>	25.6	27.2	<b>26.4</b>	23.2	34.8	<b>29.0</b>	<b>27.7 c</b>
Kuroda	24.1	25.2	<b>24.7</b>	23.4	24.6	<b>24.0</b>	25.7	29.4	<b>27.6</b>	<b>25.4 d</b>
Aladin	35.2	23.7	<b>29.5</b>	31.5	24.8	<b>28.2</b>	29.7	32.7	<b>31.2</b>	<b>29.6 b</b>
Roko	28.1	26.6	<b>27.4</b>	33.1	31.8	<b>32.5</b>	22.9	31.4	<b>27.2</b>	<b>29.0 b</b>
Desiree	29.9	22.2	<b>26.1</b>	26.7	29.2	<b>28.0</b>	27.3	25.7	<b>26.5</b>	<b>26.8 c</b>
Rudolph	37.4	28.6	<b>33.0 b</b>	33.7	30.9	<b>32.3 ab</b>	32.4	33.7	<b>33.1 a</b>	<b>32.8 a</b>
Average (B)	30.7	25.3	<b>28.0</b>	29.0	28.1	<b>28.5</b>	26.9	31.3	<b>29.1</b>	<b>28.5</b>
Average (C)	2010	<b>28.9 a</b>								
	2011	<b>28.2 b</b>								

Source	lsd	
	0.05	0.01
Variety (V)	0.9292	1.2291
Year (Y)	0.5365	0.7625
Location (L)	0.6570	0.8691
V*Y	1.3140	1.7383
Y*L	0.9292	1.2291
V*Y*L	2.2760	3.0108

Apart from individual influence of the factors, their interactions (V\*Y, Y\*L, G\*Y\*L) were also high significant for investigation trait. Analysis of interactive influence of factors studied demonstrates that statistically significant higher tuber yields were obtained in Žabljak in 2011 (31.3 t.ha<sup>-1</sup>) and Nikši in 2010 (30.7 t.ha<sup>-1</sup>) comparing to the all other locality x year interactions. Variety Rudolph had significantly higher yields in 2010 (34.5 t.ha<sup>-1</sup>) while Kuroda significantly lower yields in the same year (24.4 t.ha<sup>-1</sup>). Highest yield in the research is measured in crops with varieties Rudolph, on locality Nikši, in 2010 (37.4 t.ha<sup>-1</sup>), while the lower potato yield was obtained in variety Desiree, in Nikši, in 2011 (22.2 t.ha<sup>-1</sup>).

### Conclusion

The two-year research results of investigation of productivity of different red skin potato varieties in mountainous region of Montenegro allow us to conclude:

- The highest tuber yield had in Rudolph variety (32.8 t.ha<sup>-1</sup>), while the lowest yield was measured in the crop of Kuroda variety (25.4 t.ha<sup>-1</sup>).
- Highest productivity was in Žabljak (29.1 t.ha<sup>-1</sup>), while the lowest was in Nikši (28 t.ha<sup>-1</sup>).
- As the result of the higher total amount of rainfall higher potato yields were obtained in 2010 (28.9 t.ha<sup>-1</sup>).
- Localities and years of investigation, individually and in interaction, have had a major influence on tuber yield of potato varieties studied
- Considering the obtained yields of tubers of six potato varieties with red skin color tested, the best varieties for growing in the north of Montenegro are Rudolph, Aladin and Roko.

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## HONEY PLANTS OF FOREST LANDS IN THE NORTH-WEST RUSSIA

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### Abstract

The total area of Russia forest resources is more than 900 thousand hectares and half of those resources are suitable for the organization of forest apiaries. The studied objects were covered and uncovered with forest stand lands in the ecological condition of North-West Russia. Results of the researching shows that the number of species, their occurrence and projective cover in different land categories are significantly unequal. Biodiversity of the uncovered forest lands is always richer than under the canopy of the forest stands of any type, any structure and any crop density. Our research also shows that the species composition of honey plants depends first of all on the growth conditions and category of forest land.

**Keywords:** honey , honey plants,, apiculture, bee products.

### Introduction

More than half of Russian forest resources are suitable for the organization of forest apiaries. According to the forest inventarisation (01.01.2012), the total area of Russia forest resources is more than 900 million hectares. Russia takes first place in the world among melliferous territories. The main parts of apiaries are located on the territory that belongs to the forest lands. Honey productivity of forest lands is significantly different in different zones and areas, and the main reason is the biodiversity of melliferous plants.

In Russia, the annual honey production is more than 55 million kg (Statistical Yearbook... 2008-2011.), and the total population of Russian Federation, according to the report of population census 2012, is more than 143 million people. Therefore in average per person daily amount of honey is no more than 1 g. In many European countries, the average consumption of honey is 7-10 grams per day (Nygren et al., 2006; Wong et al., 2001; FAO, 2001). This is despite the fact that the respective resource potential of the Russia is much higher than European (Grjazkin & Smirnov, 2008).

The study objects were honey plants growing on the covered and uncovered forest lands (by honey plants we mean the plants that provide nectar and pollen)

### Material and methods

Detailed researches were carried out in North-West Russia (Leningrad region) in period from March to October 2012. At the same time we take into account the honey and beebread plants separately. Accounting of honey plants was performed by selectively statistical method: the circular areas of 10 m<sup>2</sup> were placed one by one, touching each other, in three or more different rows, throughout the experimental objects. The sample magnitude must be at least 2% of the total subject area (Grjazkin, 1997). During the empiristic works the plants were differing by life forms such as herbaceous plants, sub-shrubs, shrubs and trees. For assessing the resources of honey plants were used the existing regulations (Rules for

using of forests for agriculture ..., 2007; Manual Accounting and evaluation of secondary forest resources ..., 2003).

### Results and Discussion

Results of the study show that the number of species, their occurrence and projective cover is significantly different by land categories. On the uncovered forest lands their number is always greater than under the canopy of the forest stands of any type, any structure, and any completeness. This is due to the fact that on the glades, clearings and burnt areas there is large proportion of helophytes and ruderal species (weeds).

Accordinging the fact that the amount of honey plants is very large, a calendar of their bloom is highly extended - from March to October. Following that fact the total number of blooming species and the proportion of honey plants significantly changed monthly (table 1).

Table1. Dynamics of honey plants by month and their distribution on life forms

Months	The total number of blooming plant species	The honey plants by live forms		
		Woody plants	Semifrutexes	Herbaceous plants
March	3	3	no	no
April	11	4	no	4
May	32	2	5	12
June	54	2	2	19
July	36	1	1	15
August	28	1	1	6
September	17	no	1	5
October	3	no	1	3

The first blooming honey plants (the second or third decade of March) are represented by different types of willows. Later, in April blooming hazel (*Corylus avellana L.*) and alder (*Alnus glutinosa L.*); and from the grass cover – primrose (*Primula veris L.*), coltsfoot (*Tussilago farfara L.*). In May, the number of blooming honey plants increases several times, and there maximum number in the ecological conditions of North-West Russia was observed in June and in the first half of July. The most late blooming honey plants are white nettle (*Lamium album L.*) and red deadnettle (*Lamium purpureum L.*), tufted vetch (*Vicia cracca L.*) and common vetch (*Vicia angustifolia L.*), common knotgrass (*Polygonum aviculare L.*) and heather (*Calluna vulgaris L.*). *Calluna vulgaris* is also the longest-blooming species in the Leningrad region.

The total number of plant species accounted for the entire observation period is 160. Out of them 16 species belongs to the woody plants (only 5 species are honey plants: honeysuckle (*Lonicera xylosteum*), maple (*Acer platanoides*), linden (*Tilia europea*), mountain ash (*Sorbus aucuparia*), wild cherry (*Prunus avium*). The pollen collection carried by bees, mainly collecting from willow and hazel. The shrubs are represented by 6 species. The main part of shrubs concerns to the honey plants, and the primary honey plant from this group is the *Calluna vulgaris L.*

According to the majority of apiarists, the largest honey production from all forest grass species in the environmental conditions of North-West Russia has one species – willow-herb (*Chamerion angustifolium*). The time of blooming willow-herb is coinciding with the maximum of honey flow in whole this region.

Our research found that the species composition and biodiversity of honey plants in the first place depend of growth conditions and of the category of forest land (Gryazkin & Smirnov, 2008). Species composition of honey plants at the uncovered forest lands is more abundant than under the canopy of tree stands, regardless of their performance.

The results indicate that the largest number of honey plants is founded on the burned areas and forest clearings (table 2).

Table 2. The number of honey plant species, according to the category of land

Forest land	The total number of plant species	The number of honey plant species
1.The wood sorrel spruce forest	25	17
2. The wood sorrel birch forest	29	19
3.The bilberry pine forest	24	14
4.The bilberry scrub forest	21	12
5. The bilberry birch forest	24	15
6. Clearing 3 years ago	33	21
7. Clearing 5 years ago	38	22
8.The burned-out forest areas 5 years ago	52	25
9.The glade	50	24

This is consistent with data from other researchers (Petrick et al., 2005; Sharashkin et al., 2005; Wong et al., 2001). It is known fact that in forest apiculture is better to use clearings and burnt areas only 3 years after forest management activities or wildfire (Gryazkin & Smirnov, 2008; Petrik et al., 2005; Sharashkin et al., 2005).

### Conclusion

Research shows that the species composition of honey plants in the first place depends on growth conditions and the category of forest land. At the uncovered forest lands species composition of honey plants is more abundant than under the canopy of tree stands, irrespective of the characteristics of forest plant communities.

From the total number of recorded species, only 58 can be attributed to the honey plant (these types are visited by bees). Out of them 4 species belongs to the woody plants but the most pollen producers of them are willow and hazel. From 6 species of semifrutexes 4 refers to honey plant and the main honey plant of this group is *Calluna vulgaris* L. The largest honey production from all herbaceous plants in the condition of North-West Russia has one species – willow-herb.



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**STUDY FOR DETERMINATION OF CLIMATIC SIMILIARITES TO DIFFERENT AGRO-ECOLOGICAL ZONES OF THE ALBANIA TERRITORY**

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**Abstract**

In the environment where agricultural activity is applied, the climate is a complex specific environment, whose study is important for the stability and sustainability of agricultural production. The studies in agro-ecology, under the conditions of agricultural orientation of Albania make necessary the climate classification of agricultural areas, based on the suitability of agricultural plant groups, whose productivity level and sustainability depend on the performance and fluctuations of climatic elements. The introduction of new cultivation technologies of plants with low environmental impact requires the recognition of environmental features and in particular the climate one with the intention of satisfying the needs of each plant cultivated which is cultivated and spread in that area. To be more precise, the characterization and climatic zoning of the territory is considered to a valuable study in order to determine the most appropriate ecological zones in the country. For purposes of characterization of similar climate zones in the Albanian territory have been taken the historical series of climate data, which have been digitized and processed by applying the method "*Cluster analysis*" with the view to distinguishing their climate features, which will serve to distinguish the closely-related ecological areas and designing appropriate technologies for cultivation of plants based on such data, to increase the sustainability of agro-ecosystems.

**Key words:** temperature, precipitation, relative humidity, climatic zones, agro-ecological zones

**Introduction**

There is no doubt that in the framework of ecological conditions, the climate is a very important part of the general environment where organisms are grown, having a great impact on them. Different climate regimes play a determinant role on the geographic and agronomic distribution of plants and on the development rate of crops also. The climate, in a considerable scale, restricts the structural characteristics and particularly the rate of plants development ([Peculi V.,et al, 2007. Regarding the climate conditions the territory of Albania, belongs to the subtropical sub belt of western coasts which is divided in two climatic zones: the Atlantic Mediterranean zone and the continental zone (Hidmet. 1978).But the microclimatic effects created over the Albanian territory make the climate too varied. The determinant factors of climate are: country position versus general atmospheric circulation, latitude, relive configuration with a very broken orographic structure, the presence and differences between Ionian and Adriatic Sea and the orientation of mountainous masses. All these factors make it possible that in a relatively small area, wide changes of climatic elements are created resulting in a variety of zones with different climatic characteristics and with determinant impact on crops cultivation (Peculi V.,et al, 2006) According to the climatic characteristics of agro ecological zones and homogenous climatic zones, the agro-technologies of plant cultivation should be prepared and they should take into consideration different agricultural systems applied in these zones. The territory of our country is divided in four agro-ecological zones:

Western lowland zone, Intermediate zone, south mountainous zone and North and Central mountainous zone, with distinct differences among them regarding the climatic conditions. The determination of climatic homogenous zones within them will help in composing more suitable technologies of crops cultivation and for their yield and sustainability levels.

### **Materials and methods**

In order to find out the characteristics of similar climatic zones, analysis of series of historic data of monthly precipitation, temperatures and relative humidity, published by IHM in 1978, 1979 and 1980, (Hidmet. 1978, 1979, 1980) are accomplished. The published data refer to 228 stations distributed all over Albanian territory. Data series involve different time periods starting from 1931 to 1975. The values of climatic variables are calculated and analyzed for the precipitation data of 222 stations, the data of minimal, maximal and mean monthly temperatures for the period 1966-1975 for 147 stations. For humidity: monthly data of number of days with relative humidity 50% and 80% during 14 hours in 45 stations (Hidmet. 1980). For the data groups a Cluster analysis was applied aiming the determination of similar climatic zones. “*Cluster analysis*” is a technique that allows creating of groups (clusters) in a way that the elements belonging to the same group are as homogeneous as possible among them, while the elements belonging to different groups should be heterogeneous (Hartigan JA, 1975). Given that the data are not hierarchical, the procedure of “*fastclus*” is applied according to Sas Institute Inc, (1987). To determine in a simple way the periods with droughts in each homogenous climatic zone the data are elaborated according to the method of ombrothermic diagrams Bagnlous & Gossen (Bagnlous F, 1957).

The data taken from the elaboration made to determine the homogenous climatic zones are compared with the division of agro-ecological zones trying to find similarities and differences for each zone.

### **Results and discussions**

Albania is characterized by high values of precipitations which are in between of an interval from 649 mm to 1060 mm per year in lowland and hilly zones including the districts of Vlorë, Fier, Berat, Lushnjë, Durrës; that belong to the agro ecological zone of Western lowland, in South east we have the districts of Kolonjë, Korçë and Pogradec (South mountainous agro-ecological zone) (Shundi A, 2003), and in North East we have the districts of Dibra and Kukës (North and Central mountainous zone) (Shundi A, 2003). In these zones we have similar climatic areas noted with numbers 2,6,10,11,12,13,20 and 25 (Figure 1) (INTERREG, 2001); and maximal values included in the interval from 2295 to 3117 mm per year, mainly in South mountainous zones in the districts of Gjirokastër, Tepelenë (South mountainous agro ecological zone) and Vlorë and Sarandë (Intermediate agro ecological zone) (Shundi A, 2003); from the North mountainous agro ecological zone here we have the districts of Puke, Tropoje e Shkoder that are in similar climatic areas and have the numbers 5 (in South) and 3 and 18 (North). We could see that in different agro-ecological zones we find similar climatic areas.

The mean annual temperatures vary between 13.9 and 17.1<sup>0</sup>C in lowland and hilly zones along the coastline and between 7.5 and 10.7<sup>0</sup>C, in hilly and mountainous zones in the inner part of the country. The climatic zones with higher annual mean temperatures included between 14.4 and 16.3<sup>0</sup>C are marked with numbers 1,5,6,10,14,17,20,22 and 24 placed along coastline; the annual maximal mean temperature of these areas are between 19.2 and 21.3<sup>0</sup>C while the mean minimal temperatures are between 8.6 and 11.2<sup>0</sup>C. The zones with lower annual mean temperatures included between 9.1 and 10.7<sup>0</sup>C are in the mountainous South East and Northern part of the country, marked with numbers 2,3,4,13,15 and 25 belonging to

the districts of Shkodra, Pukë, Kukësi, Dibra, Korça and Kolonja, the mean maximal temperatures of which are between 13.3 and 15.6<sup>0</sup>C and those minimal are between 4.4 and 6.0<sup>0</sup>C.

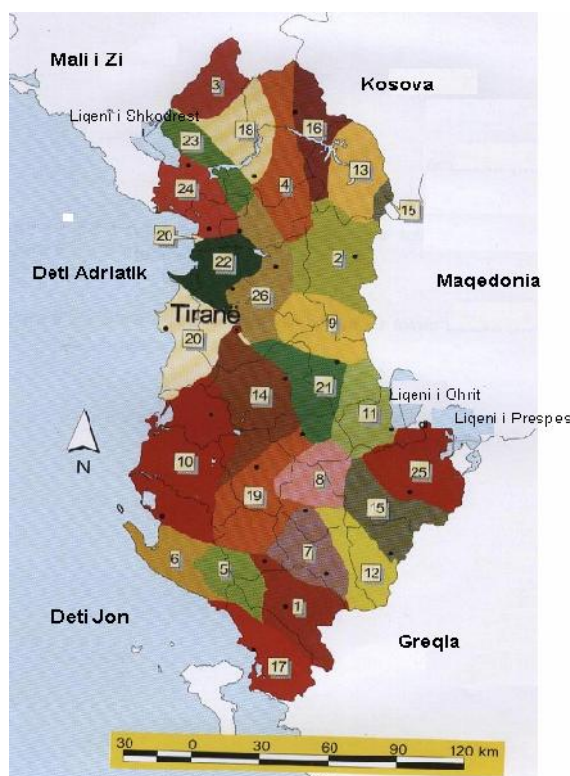


Figure 1. Homogeneous climatic zones of Albania [10]

As regards the relative humidity, two variables are evaluated: monthly and annual number of days with relative air humidity >80% for at least 14 hours per day and the number of days with air relative humidity <50%. The zones with higher number of days (82-98) with air relative humidity >80% and a relatively low number of days (124-143) with humidity <50% are found in the districts of Shkodra, Tiranë, Matit, Dibra and Librazhdi; the similar climatic areas of in these zones are marked with 7,12 and 14 (Figure 1). The monthly and annual values of parameters that characterize each cluster are shown in table 1.

Table 1. The values of climatic parameters according to the homogenous climatic zones

<b>Homogenous climatic zones</b>	<b>Average annual number of days with humidity &gt;80% for 14 hours</b>	<b>Average annual number of days with humidity &lt; 50% for 14 hours</b>	<b>Annual mean temperature (°C)</b>	<b>Annual precipitation (mm)</b>
1	42,8	182,8	8,7	1858
2	68,3	151,9	5,3	1172
3	91,7	139,2	5,2	2537
4	73,8	143,7	6,0	1942
5	40,1	164,3	10,4	2039
6	28,5	152,2	10,8	1732
7	41,8	204,2	8,1	1328
8	64,1	171,5	7,2	1080
9	82,4	143,7	6,7	1254
10	30,7	160,3	10,2	805
11	60,3	144,4	6,3	1101
12	42,9	197,0	6,2	1207
13	56,1	171,0	5,5	1091
14	37,8	174,4	9,5	1031
15	53,6	171,1	4,4	967
16	65,1	154,6	6,2	1514
17	32,2	163,8	11,2	1445
18	81,9	137,8	6,9	2719
19	51,0	175,1	9,3	1116
20	44,6	139,0	10,0	1151
21	58,2	160,7	7,8	1656
22	57,2	133,4	9,7	1314
23	72,0	140,9	9,3	1953
24	52,5	145,5	11,1	1751
25	58,5	155,4	4,7	806
26	59,9	142,9	8,1	1648

Drought periods in homogenous climatic zones are determined through the method of ombrothermic diagrams Bagnlous & Gossen. Analyzing the data from the construction of ombrothermic diagrams it results that drought periods generally are short all over the territory of Albania and they happen during July and August. These periods are more evident in the South mountainous zone and Intermediate zone along the coastline which belong to the similar climatic zones 1,5,6,7,10,12,14,17,19 and 20.

After analyzing the data, the output regarding determination of similar climatic zones is compared with the division of agro ecological zones trying to find both differences and similarities. This will provide the possibility of differentiating the cropping technologies according to these differences.

The territory of our country is divided into four agro ecological zones: Western lowland zone, Intermediate zone, Southern mountainous zone and Northern and Central mountainous zone (Shundi A, 2003). These zones have different ecological characteristics. The Western lowland zone is placed along the coastline of Adriatic Sea and includes the districts of Durrës, Fier, Kavajë, Laç, Kuçovë, Lezhë, Lushnjë and Peqin, with mean annual temperature 14 – 18<sup>0</sup>C; during summer time in Southern zones its value is around 26<sup>0</sup>C, while winter temperatures have an average of 9.8<sup>0</sup>C. The northern part of this zone is a little colder with mean summer temperatures of 23.5<sup>0</sup>C. The mean annual precipitations are 800 – 1200 mm and 80% of them are during the period October-March. The Northern parts are exposed to a high risk of frost during winter months (Shundi A, 2003). From this study we can see that some districts that belong to different agro ecological zones have particular parts belonging to similar climatic zones regarding the level of precipitation, temperatures and air relative humidity.

The intermediate zone, a transition hilly zone between the coast lowland zone in West and the mountainous zone in East is placed starting from the most southern point of the country to the most northern part. Regarding elevation this zone varies from 100m to 900m above sea level and includes parts of the districts: Berat, Delvinë, Elbasan, Krujë, Mallakastër, Sarandë, Shkodër, Tiranë, and Vlorë. The mean annual level 5 – 16<sup>0</sup>C. The mean annual rainfall 800 – 2500 mm. Precipitation dominate during winter and generally are higher in northern and southern districts, while the districts of the central part are drier. This zone is exposed to high frequency of frost, especially during winter time (Shundi A, 2003).

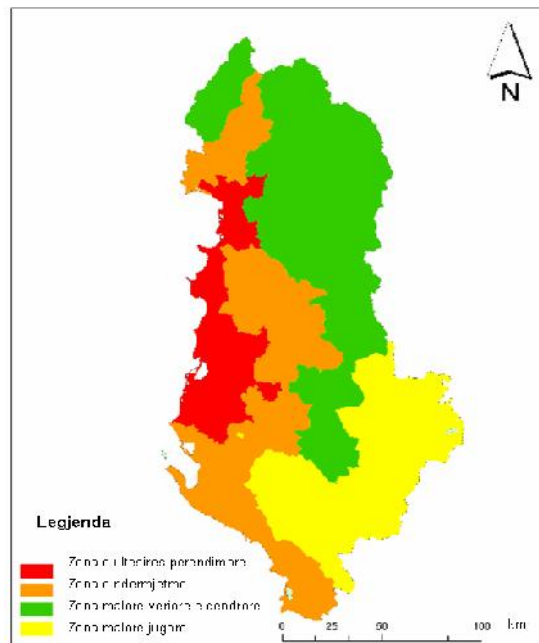


Figure 2. The agro ecological zones of Albania (Source: Shundi, A. 2003. “Country profile for pastures/ forages” – Albania, FAO)

The South mountainous zone lies in the most part of the South part of Albania. It includes the districts: Devoll, Korçë, Kolonjë, Përmet, Pogradec, Tepelenë, and Gjirokastër. Mean annual temperature is 6 – 14<sup>0</sup>C, during summer time 18-20<sup>0</sup>C and during winter time the

average temperature is under 10 °C. Mean annual rainfall is from 600 to 1200 mm. The frosts in this zone limit the agricultural production (Shundi A, 2003).

The Northern and Central zone includes the districts: Bulqizë, Dibër, Gramsh, Has, Kukës, Librazhd, Malësi e Madhe, Mat, Mirditë, Pukë, Skrapar and Tropojë. The mean annual temperature is 3 – 12 °C. Maximal temperatures reach 25 °C in July, while the minimal temperatures go to –20 °C. Mean annual rainfall is from 800 to 2500 mm and they are mainly during winter time. The zone is exposed to high quantities of snow and number of frost days (Shundi A, 2003).

By comparing the data it results that in the four agro ecological zones we find similar climatic areas that provide the opportunity to prepare more suitable crop technologies with low environmental impact. In order to have a more detailed view of the characteristics of homogenous climatic zones, an analysis of the main components; level of rainfall, temperature and air humidity is done through the method “*varimax*” according to SAS/STAT. From this analysis it resulted that quantity of rainfall and temperature determine the major part of the total climatic variability (about 80 %). The quantity of precipitation is correlated positively with minimal and maximal temperatures during all months of the year and negatively with the values of relative air humidity during winter months, while temperature is correlated positively with the values of rainfall quantity during all months of a year and with the values of relative air humidity during the period April-November.

### Conclusions

From analyzing the data of Albanian territory 26 similar climatic zones have resulted. They lay in different agro ecological zones. Similar climatic areas with lower quantity of rainfall (where the rainfall with 75% probability are from 694 to 968 mm and with 25 % probability are from 944 to 1396 mm) are marked with numbers 2,8,10,11,12,13,15,19,20 and 25 and belong to the districts of Kukës, Dibër, Pogradec, Korçë and Kolonjë of the mountainous northern and central zone; Fier, Lushnje, Durrës and Tiranë from the western lowland zone and Tepelenë, Berat and Skrapar in the intermediate zone. Similar climatic areas with higher quantity of rainfall (where the rainfall with 75% probability are from 1397 to 2099mm and with 25 % probability are from 2048 to 3214 mm) are marked with numbers 1, 3, 4, 5, 6, 18, 23, 24 and includes parts of the districts Shkodër, Tropojë and Pukë in the northern and central mountainous zone and Vlorë, Tepelenë and Gjirokastër in the intermediate and southern mountainous zone.

Similar climatic areas with high temperature (maximal absolute), hot areas, areas with mean annual temperatures from 14,4 to 16,3 °C, marked with numbers 1,5,6,10,14,17,20,22 and 24 are those registered in July (the temperatures with 75% probability is from 32 to 33,9 °C and with 25 % probability is from 35,6 to 37,4 °C). Minimal absolute temperature with 75% probability is from -2,3 to 5,6 °C.

Similar climatic areas with low temperatures, cold zones with mean annual temperature from 9,1 to 10,7 °C marked with numbers 2,3,4,13,15,25 belong to districts of Shkoder, Pukë, Kukësi, Dibër, Korçë and Kolonjë, the maximal temperature of which is from 13,3 to 15,6 °C and those minimal are from 4,4 to 6,0 °C. In these areas the maximal absolute temperatures are in July and with a probability of 75% are between 27,3 and 31 °C and with probability 25 % are between 30,5 and 34,6 °C, the minimal absolute temperature in January and February with probability of 75% are between -5,9 and -7,9 °C, with 25% probability are between -11,6 and -14 °C.

Regarding the relative air humidity we have low values of relative humidity in south areas and high values in North of Albania. The drought periods generally are short all over Albania and they happen during July and August. They are more severe in southern

mountainous and intermediate zones, along coastline and belong to the climatic homogenous zones 1,5,6,7,10,12,14,17,19 and 20 (Figure 1) (INTERREG 2001).

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## TOTAL AND ORGANIC PHOSPHORUS STATUS IN SOILS OF EASTERN CROATIA

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### Abstract

Far as is known there is no relevant information about the status of total and organic phosphorus in Croatian soils, so aim of this study was to determine the total and organic phosphorus in soils of the eastern Croatia. In total were collected 94 soil samples and the soil pH, organic matter as well as the total phosphorus content and organic phosphorus content were analyzed. All samples were grouped according to soil pH and organic matter in two groups ( $\text{pH}_{\text{KCl}} < 6$ ,  $\text{pH}_{\text{KCl}} > 6$ , organic matter  $< 2\%$ , organic matter  $> 2\%$ ). Analyzed samples showed significant amounts of total phosphorus with minimal content of  $329.84 \text{ mg P}_2\text{O}_5 \text{ kg}^{-1}$ , while the maximum value determined by the total phosphorus was  $1732.19 \text{ mg P}_2\text{O}_5 \text{ kg}^{-1}$  with an average of  $713.03 \text{ mg P}_2\text{O}_5 \text{ kg}^{-1}$ . The content of organic phosphorus in soils ranged from  $3.44 \text{ mg P}_2\text{O}_5 \text{ kg}^{-1}$  to  $292.55 \text{ mg P}_2\text{O}_5 \text{ kg}^{-1}$ , with the portion of organic phosphorus in total phosphorus from 0.54% to 78.29%. Seen from the humus content in the soil, very low humic soil had an average of 12.0% of organic phosphorus, while fairly humic soil had an average of 7.1% organic phosphorus. Also, the results showed that the minimum (0.54%) and maximum (78.29%) portion of organic phosphorus in total phosphorus soils recorded within broad categories humic poor soils. Although it was expected, there was no significant differences between the amount of organic phosphorus in soil and organic matter content, but large influence of organic matter content to the correlation of certain fractions of phosphorus in the soil were determined ( $r = 0.89$ ).

**Key words:** soil, total phosphorus, organic phosphorus, portion

### Introduction

Total phosphorus are all fractions of phosphorus that exist in the soil and all organic and inorganic forms of phosphorus and oxidation of organic compounds and inorganic dissolution by acids necessary to determine the total amount of phosphorus in the soil. Many authors point out that there is an essential difference between the total and plant available phosphorus in the soil. Total phosphorus can be successfully predicted from the content of humus and common extraction methods such as Olsen or Bray (Bundy & Good, 2003) and few parameters are important for predicting the total phosphorus in the soil: the physical properties of the soil, the amount of water in soil chemical properties and organic matter (Ulen et al., 2000).

In most agricultural soils, organic P comprises 30-80 % of total P. The largest fraction of organic P over 50 % appears to be in form of phytin and its derivate (Tarafdar & Claassen, 1988). The significance of these organic P compounds for the P nutrition of plants is not clear. Generally, it is assumed that plants take up P as inorganic phosphate from the soil solution and than phosphatases, either from plant root or soil microorganisms, must first hydrolyze organic P compounds in soil (Hayes et al, 2000).

### Materials and methods

In total were collected 94 soil samples from eastern Croatia and the soil pH (ISO 10390), organic matter (ISO 14235) as well as the total phosphorus content (ISO 11466) and organic phosphorus content (Kuo, 1996.) were analyzed. All samples were grouped according to soil pH and organic matter in two groups and several subgroups ( $\text{pH}_{\text{KCl}} < 6$ ,  $\text{pH}_{\text{KCl}} > 6$ , organic matter  $< 2\%$ , organic matter  $> 2\%$ ). Total organic phosphorus determined by annealing samples. The method is based on the annealing process, which translates into an organic phosphorus inorganic form, and then determines the total amount of organic phosphorus by comparing the amount of phosphorus in the sample inorganic with or without prior annealing. Concentration of total phosphorus in both samples (with or without annealing) is calculated by the equation:

$$\text{Total P (mg kg}^{-1}\text{)} = \text{P conc (mg ml}^{-1}\text{)} \times 50 / \text{g}$$

g = weighs soil

P = total organic P annealing - total P without annealing

Total phosphorus in the soil was determined destruction of soil samples with aqua regia (ISO 11466, 1995). Concentration of total phosphorus was determined by ICP-OES technique (induced coupled plasma-optical emission spectrometry) and expressed as  $\text{mg P l}^{-1}$ . The result then was converted into  $\text{mg P}_2\text{O}_5 \text{ kg}^{-1}$ .

### Results and discussion

Total phosphorus in soil consists of organic and inorganic forms of phosphorus, and considering that the method for determination of total phosphorus in the soil complicated analyzes were made on 94 samples, primarily to study the relationship between the total, organic and inorganic phosphorus in the soil. Criteria for samples distributions was  $\text{pH}_{\text{KCl}}$ , (Figure 1).

Highly acidic soil ( $\text{pH}_{\text{KCl}} < 4$ ) were presented with the lowest number of samples (13), and most of the samples were from a group of slightly acidic soils (23).

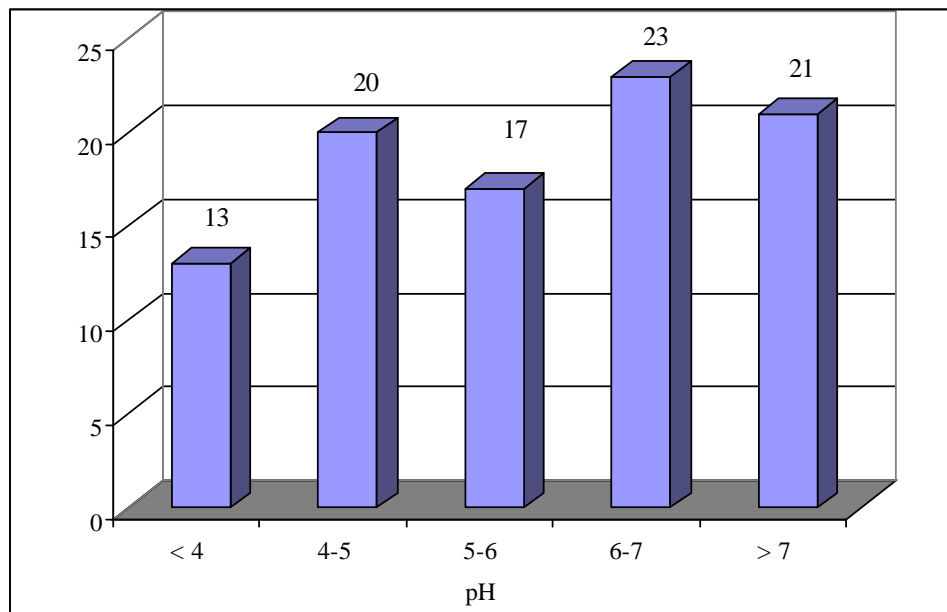


Figure 1. Soil samples distribution according to  $\text{pH}_{\text{KCl}}$

In the analyzed samples showed significant amounts of total phosphorus from 329.84 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup>, to 1732.19 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> with an average value of 713.03 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> (Table 1).

Table 1. Minimum, maximum and average of total and organic P

<b>Total P<sub>2</sub>O<sub>5</sub> mgkg<sup>-1</sup></b>		<b>Organic P<sub>2</sub>O<sub>5</sub> mgkg<sup>-1</sup></b>	
Min	329,84	Min	3,44
Max	1732,19	Max	292,55
Average	713,03	Average	56,9

There was no significant differences between the minimum, maximum and average values of total phosphorus in soils according to different acidity (Table 2). However, the lowest total phosphorus was determined in slightly alkaline soils and followed by a total phosphorus in highly acidic soils.

Table 2. Minimum, maximum and average of total and organic P in different range of soil pH

<b>Total P<sub>2</sub>O<sub>5</sub> mgkg<sup>-1</sup></b>				<b>Organic -P<sub>2</sub>O<sub>5</sub> mgkg<sup>-1</sup></b>			
<b>pH<sub>KCl</sub></b>	<b>min</b>	<b>max</b>	<b>average</b>	<b>pH<sub>KCl</sub></b>	<b>min</b>	<b>max</b>	<b>average</b>
<4	373,69	1211,23	669,45	<4	6,3	292,55	81,69
4-5	457,06	1254,95	742,38	4-5	4,58	188,93	45,86
5-6	429,26	1175,39	724,21	5-6	3,44	201,52	62,77
6-7	408,09	1264,93	723,06	6-7	4,58	156,87	54,09
>7	329,84	1732,19	692,03	>7	11,45	237,59	50,38

Many authors point out that there is an essential difference between the total and plant available phosphorus in the soil (Al-Jaloud et al. 1998; Kuo, 1996). As proved by Bundy and Good (2003) total phosphorus can be successfully predicted from the organic matter content and common extraction methods such as Olsen or Bray. Four parameters are important for predicting the total phosphorus in the soil: the physical properties of the soil, the amount of water in soil, chemical properties and organic matter content (Ulen et al., 2000.).

As well as total phosphorus, in the same number of samples organic phosphorus in the soil were determined and the criteria for selecting samples were organic matter content (<2% and > 2%) and soil pH.

The determined value of organic phosphorus in soils ranged from 3.44 to 292.55 mg kg<sup>-1</sup> with an average of 56.9 mg kg<sup>-1</sup> (Table 1). Distribution of samples at pH values showed that, on average, the highest concentration of organic phosphorus were represented in the group of highly acidic soils (81.69 mg kg<sup>-1</sup>) while in other groups were occurred approximately the same value of organic phosphorus (Table 2).

Considering the organic matter content samples were divided into six groups: <1% organic matter, 1.0-1.5% organic matter, 1.5-2.0% organic matter 2.0-2.5% organic matter, 2,5 - 3.0% organic matter and > 3% organic matter. According to Gra anin these groups can be categorized into three: very low humic soil (<1%), low humic soil (from 4 categories by previous division: 1-3%), and plenty of humic soil (> 3%) (Figure 2).

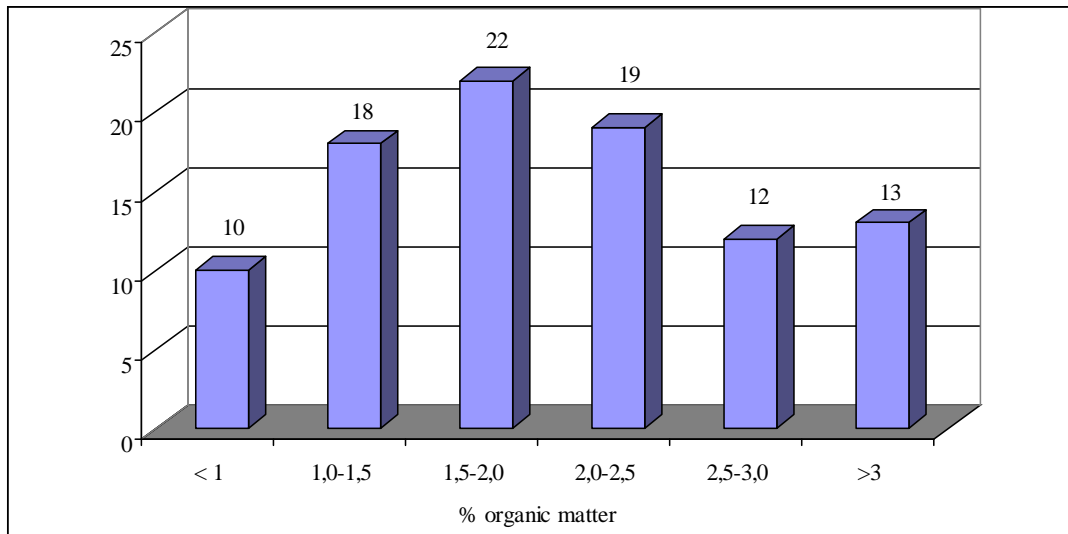


Figure 2. Soil samples distribution according to organic matter content

The share of organic phosphorus in total phosphorus were ranged from 0.54% to 78.29%. The minimum proportion of organic phosphorus in total phosphorus was recorded in the sample from the group moderately acidic soils ( $pH_{KCl}$  5-6), while the maximum share recorded in samples from a group of highly acidic soils ( $pH_{KCl}$  <4). The soils of extremely acidic reaction was recorded the highest (14.32%) average share of organic phosphorus in total phosphorus (Figure 3). Generally, the minimum registered share of organic phosphorus in total phosphorus was in slightly humic moderately acidic soils (0,54 %), while the maximum share was recorded in extremely low humic acid soils (78,29%).

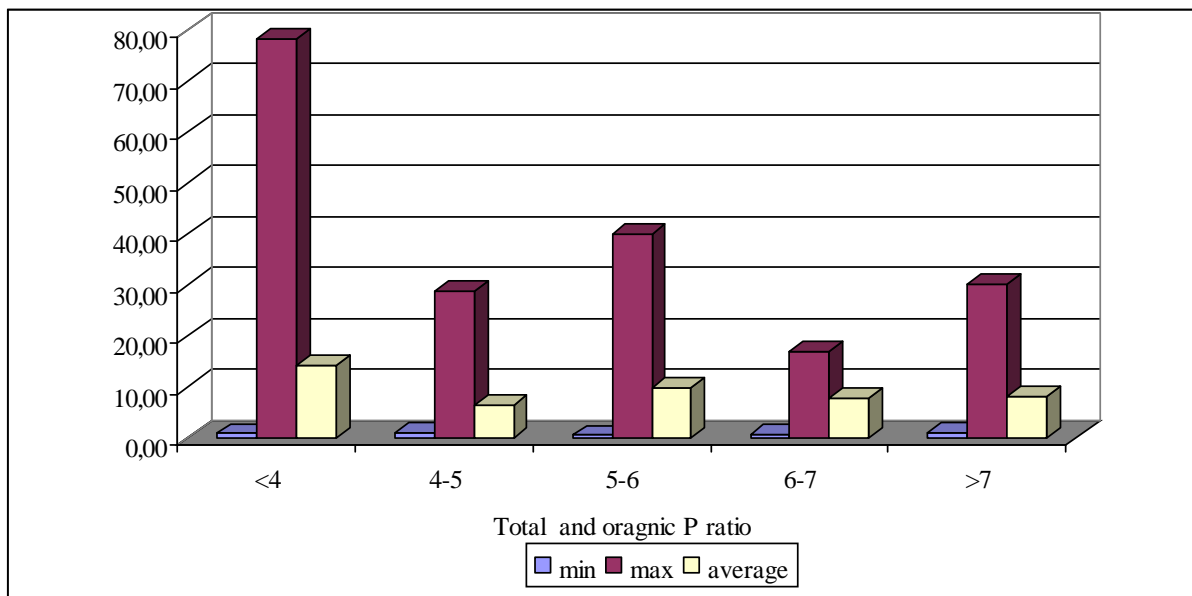


Figure 3. Total and organic P share

In most soils, organic phosphorus is more than 50% of total phosphorus, mostly in the form of inositol phosphate penta and hexa-related iron and aluminum (Borie & Rubio, 2003). Since most plants can intake phosphorus exclusively in inorganic form enzyme phosphatase activity in the soil are crucial.

### Conclusion

pH reaction had no effect on the concentration of total and organic phosphorus in the eastern Croatia soils. Also, although the amount of organic phosphorus in soil is closely related to the amount of organic matter, expected results of their correlation were absent, but the organic matter content significantly influenced the simplified fraction of phosphorus in the soil ( $r=0,89$ ). Seen from the organic matter content in the soil, very low humic soil had an average of 12.0% of organic phosphorus, while fairly humic soil had an average of 7.1% organic phosphorus. Different proportion of organic phosphorus in very low humic soils in relation to humic soil, probably due to varying intensity of mineralization, C / P, N / P ratio, and different enzyme phosphatase activities. Also, the increase in organic matter content of the soil can be binding free inorganic phosphorus (Humate effect) affected by the decreased in the share of organic phosphorus in the soil.

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**A STUDY OF DIFFERENT GERMINATION MEDIA FOR THE 'ŠAMPION'  
WALNUT CULTIVAR POLLEN**

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**Abstract**

Different culture media were tested in an attempt to improve the knowledge of the most suitable germination media for studying the *in vitro* pollen germination capacity of 'Šampion', a Serbian walnut cultivar. The research was conducted on agar media, as a four-factorial experiment, with different concentrations of agar (0.6%, 0.8% and 1%), sucrose (10%, 15% and 20%), H<sub>3</sub>BO<sub>3</sub> (0 ppm, 200 ppm and 400 ppm) and CaCl<sub>2</sub> (0 and 50 ppm). Pollen germination was maximized (39%) when the germination medium contained 0.8% agar, 15% sucrose, 400 ppm H<sub>3</sub>BO<sub>3</sub> and 50 ppm CaCl<sub>2</sub>. Large and significant differences in pollen germination were observed in response to changing concentrations of agar, sucrose, boric acid and calcium chloride, and strong interaction was identified between all substances used.

**Keywords:** walnut, pollen, germination medium.

**Introduction**

Information on pollen viability and germination is important for the study of the reproductive biology of the walnut and for the development of its genetic crop program. Various methods can be used for estimation of pollen viability and germinability in horticultural crops. Two basically different approaches can be taken to estimate pollen viability: staining pollen with dyes and *in vitro* germination assay. Staining techniques aim to determine pollen enzymatic activity and membrane integrity. *In vitro* germination determines the actual germination ability of pollen under suitable conditions (Shivanna et al., 1991.; Dantas et al., 2005; and Tuinstra and Wedel, 2000), and it is the most widely used method of testing pollen viability in breeding programs (Marcellán and Camadro, 1996). The composition of the germination medium can dramatically affect pollen metabolism (Taylor, Hepler 1997). Different culture media for the *in vitro* germination of pollen grains have been reported for a large number of species, with considerable variations within and among species (Pfahler et al. 1997). However, there is no reliable information about the ideal culture medium for *in vitro* testing of the walnut pollen viability. Pollen of walnut has been considered difficult to germinate *in vitro* and it requires sucrose, boron and calcium as the necessary components of the culture medium for germination and pollen tube growth (Griggs et al. 1971).

The objective of this study was to test the effect of different contents of agar, sucrose, boric acid and CaCl<sub>2</sub> in germination medium on 'Šampion' walnut pollen germination *in vitro*.

## Material and Methods

This study was carried out in the year 2010 on walnut cultivar 'Šampion'. Samples of pollen were collected near Kraljevo (central Serbia) in the morning, between 8:00 and 9:00 a.m., at the time when the first staminate flowers of the catkins had begun to shed their pollen grains. Catkins were brought into the laboratory and laid on clean black paper. The catkins were kept under the laboratory conditions to shed their pollens for 1-2 hours. The experiment was set up as a 3 x 3 x 3 x 2 factorial design with concentrations of agar, sucrose, boric acid and calcium chloride as independent variables. Concentration ranges investigated were: agar – 0,6%, 0,8% and 1%, sucrose - 10%, 15% and 20%, H<sub>3</sub>BO<sub>3</sub> - 0 ppm, 200 ppm and 400 ppm and CaCl<sub>2</sub> - 0 and 50 ppm). A total of 54 combinations of germination media were tested. The pollen samples were germinated in 35-mm sterile Petri dishes, each containing three ml of prepared germination medium. Before the deposition of the pollen onto the agar, the Petri dishes with agar are needed to be aged for at least 24 hours. If this is not done, the pollen grains tend either to sink into the agar, where it will not germinate, or to take up excessive moisture and rupture (Taylor, 1972). A fine paint brush was used to deposit the pollen on the surface of the agar in a Petri dish in order to promote a uniform distribution of the material. This is important, as agglomeration of pollen grains results in higher pollen germination (Giulivo and Ramina, 1974). The Petri dishes planted with pollen were incubated at 22°C in dark conditions. Pollen germination was arrested after 24 h by immediate freezing at -20°C. This procedure has been shown to be a highly efficient method to arrest pollen germination while preserving the material for further evaluation. One day before observation under the microscope, the frozen Petri dishes were thawed at 4°C (Hendley et al., 2005). Pollen germination was observed using an optical microscope at a 100x magnification, with approximately 20-50 pollen grains per field. The number of pollen grains counted per dish was approximately 400-600. A pollen grain was considered to be germinated when the length of pollen tube was equal to or exceeded its diameter. Fifteen different fields of vision were examined per dish. Each count was considered as one replicate. Germination percentage was determined by dividing the number of germinated pollen grains per field of view by the total number of pollen grains per field of view. A four-way analysis of variance was performed. Means were separated by Tukey's multiple range test at *P* 0,05.

## Results and discussion

The total average germination percentage of the 'Šampion' walnut pollen was 16,4%. The germination rate was maximized (39%) when the germination medium contained 0.8% agar, 15% sucrose, 400 ppm H<sub>3</sub>BO<sub>3</sub> and 50 ppm CaCl<sub>2</sub>. The analysis of variance of experimental data showed the significant effects on pollen germination of agar, sucrose, boric acid, calcium chloride and their interactions (Table 1).

### *Effect of agar*

Pollen of walnut cultivar 'Šampion' germinated significantly better on the media containing 0.8% agar (19,1%) than on the media with 0,6% and 1% agar (15,0% and 15,1%, respectively) (Table 1). There were no significant germination differences between the media with 0,6% and 1% agar. These results are consistent to the findings by Cerovi et al. (1992), who obtained the best germination of the walnut pollen on the medium with 0,75% agar. According to Luza and Polito (1985) the medium containing 0,65% agar was suitable for freshly collected pollen for each of the 21 tested walnut clones. The agar content appears to be important in providing the necessary conditions for good hydration and germination of the

walnut pollen (Luza and Polito, 1985). The concentration of agar, by changing the physical characteristics of the medium, determines the embedding degree of the grains into the surface which may affect the amount of oxygen absorbed by the grain (Visser, 1955). When lower concentrations of agar are used, the pollen tends to sink into the surface of the medium where it will not germinate.

#### *Effect of sucrose*

The germination of the pollen was also affected by sucrose concentrations, and media containing a 20% sucrose concentration had the highest average germination rate (18,2%), while there were no significant germination differences between the media with 10% and 15% sucrose (Table 1). Sütyemez (2007) found that a 15% sucrose concentration gave the highest germination rates for walnut cultivars, but according to Wu et al. (2008), the germination rate of ‘Yunxin’ walnut pollen was the highest when the sucrose concentration in germination medium was 10%. In our study, high concentration of sucrose (20%) in combination with high concentration of agar (1%) exhibited a detrimental effect on pollen germination (Graf. 1-a).

Table 1 – Effect of agar, boric acid, sucrose and calcium chloride on ‘Šampion’ walnut pollen germination *in vitro*

Factor	Concentration	Pollen germination (%)
Agar (A)	0,6%	15,0 a
	0,8%	19,1 b
	1%	15,1 a
Boric Acid (B)	0 ppm	9,1 a
	200 ppm	16,9 b
	400 ppm	23,2 c
Calcium chloride (C)	0 ppm	15,7 a
	50 ppm	17,1 b
Sucrose (S)	10%	15,7 a
	15%	15,3 a
	20%	18,2 b
Total		16,4

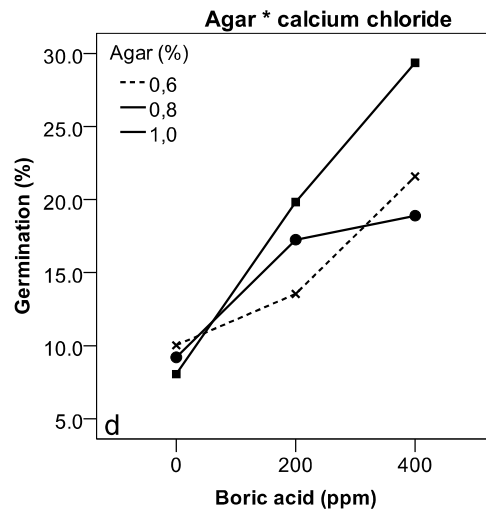
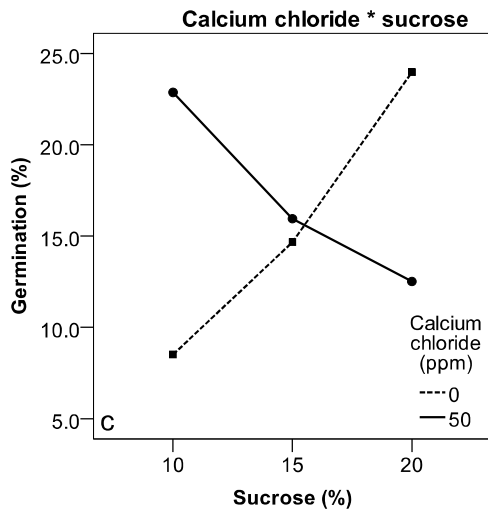
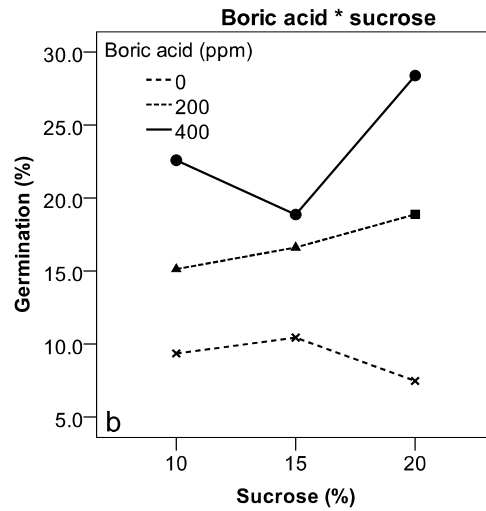
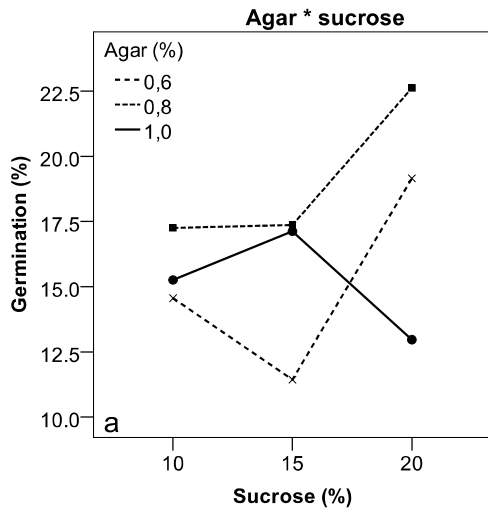
#### ANOVA

Factor	<i>p</i>
Agar (A)	0,000
Boric acid (B)	0,000
Calcium chloride (C)	0,006
Sucrose (S)	0,000
A*B	0,000
A*C	0,000
A*S	0,000
B*C	0,000
B*S	0,000
C*S	0,000
A*B*C	0,003
A*B*S	0,000
A*C*S	0,000



B*C*S	0,000
A*B*C*S	0,000

The sucrose addition to the germination medium has the objective of providing osmotic equilibrium between the pollen and the germination medium, as well as being an energy source to aid the pollen development process (Stanley and Linskens 1974). Silva et al. (1999) stated that osmotic equilibrium between the germination medium and pollen grain content determines the cell integrity, and this equilibrium can be determined by the relation between the concentration of sucrose and of substances such as boric acid and calcium.



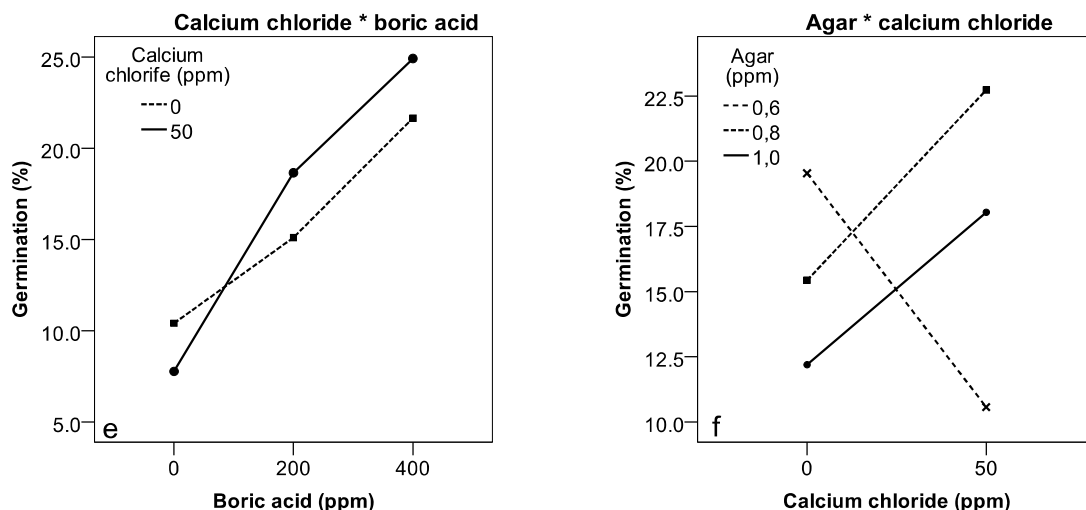


Figure 1 – Graphs showing effect of different concentration of media on pollen germination.

An excess or deficiency of any of these components could cause the breaking of the pollen grains. In our study pollen germination in media containing 20% sucrose was promoted by a high concentration of boric acid (400 ppm), but in absence of boric acid, the highest concentration of sucrose resulted in a significant decline of the germination percentage (Figure 1-b). In the media without calcium chloride pollen germination was stimulated with the increase in sucrose concentration, but in combination with  $\text{CaCl}_2$  the germination decreased (Figure 1-c).

#### *Effect of boric acid*

Adding boric acid to the substrate generally has a significant positive effect on pollen germination, but the germination increase was significantly lower in the media with the highest content of agar (1%) than in the media with a lower content of it (Figure 1–d). Wu et al. (2008) found that the optimal culture medium for ‘Yunxin’ walnut pollen contained 10 mg/L of boric acid. Boron interacts with sugar, giving origin to a sugar-borate complex (Pfahler 1967). Boron facilitates sugar uptake and has a role in pectin production in the pollen tube (Richards, 1986), thus it is indirectly involved in development of pollen tube membrane (Stanley and Loewus, 1964). According to Vasil (1960), the role of boron in pollen germination and pollen tube growth may be 3-fold: (1) it promotes absorption of sugars (2) it increases oxygen uptake and (3) it is involved in the synthesis of pectic material for the wall of actively growing pollen tube.

#### *Effect of calcium chloride*

Adding calcium chloride to the media with 0,8% and 1% agar significantly increased the germination capacity of the ‘Šampion’ pollen, while in media containing 0,6% agar the addition of  $\text{CaCl}_2$  sharply reduced the germination (Figure 1-f). The addition of calcium chloride in the media without boric acid, slightly reduced the pollen germination rate (Figure 1-e). Wu et al. (2008) noted that optimal culture medium contained 40 mg/L  $\text{CaCl}_2$ . According to Steer (1989), calcium ions are essential for pollen tube growth, but they are inhibitory at the concentrations higher than  $10^{-2}$  M.

## Conclusion

Significant differences in germination rate of the ‘Šampion walnut pollen were observed in response to changing concentrations of agar, sucrose, boric acid and calcium chloride in the germination medium. Strong interactions were identified between all substances used. The germination rate was maximized (39%) when the germination medium contained 0.8% agar, 15% sucrose, 400 ppm H<sub>3</sub>BO<sub>3</sub> and 50 ppm CaCl<sub>2</sub>.

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**EFFECT OF SLOW-RELEASE NITROGEN FERTILIZERS ON MAIZE PLANTS  
GROWN ON NEW RECLAIMED SOIL**

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**Abstract**

A field experiments was done in summer 2009 season at private farm, located at El-Sadat district, Minufiya Governorate, Egypt to evaluate the effect of ureaform or urea+humic acid as slow release nitrogen fertilizers at a rate of 60 and 100 kg fed-1 compared to urea at rate of 120 kg fed-1 on maize (*Zea mays* L.) (Single-cross 10) grown on sandy soil. The obtained results indicated that ear length, plant high, 100-grain weight, shoot and grain yields and biological yield were markedly significantly higher when application of ureaform at rate of 100 kg N/fed followed urea at rate of 100 kg N/fed + humic acid. Application of ureaform at high rate increased the values of nitrogen uptake by both shoot and grain of maize plant, while urea at high rate + humic acid induced the highest values of both phosphorus and potassium uptake for the same mentioned organs. Also, the results indicated that, maize plants received urea+humic acid or ureaform registred the highest values of fertilizer use efficiency, i.e., highest Agronomic efficiency and Apparent N recovery were obtained due to application of 60 kg N/fed urea+humic acid, while ureaform at rate of 100 kg N/fed gave the highest value of Physiological efficiency.

**Key words:** Slow release fertilizers, Sandy soils, Maize yield, Efficiency

**Introduction**

Maize (*Zea mays* L.) is one of the most strategically cereal crop grown in Egypt. Maize is the most widely cultivated cereal in the world after wheat and rice. It has a great significance as human food, animal feed and raw material source of large number of industrial products. Growing maize on newly reclaimed soils of Egypt is faced by many problems, the most important of these is the leaching of the applied N led to reduce the uptake efficiency of the addition nitrogen fertilizers by crops and is an agricultural and environmental problem.

Nitrogen is one of the most important mineral nutrient elements for existence and maintenance of higher plants life on this planet. Its main functions are serving as the constituents of many important organic compounds and participating in many essential metabolic processes in plants. Building amino acids, proteins, carriers, enzymes, regulators, nucleic acids, pigments, alkaloids and many other metabolites involve nitrogen for their biosynthesis and interconversions (Marschne 1997; Srivastava & Singh, 1999).

Nitrogen use efficiency is of significant importance in crop production system due to its impact on farmer economic outcomes and environmental impact. Nitrogen use efficiency also, may be reduced in crop production due to many factors including losses of soil nitrogen by volatilization, leaching and denitification. Jokela and Randall (1989) conducted a study of the effects of N application rate on residual NO<sub>3</sub>-N in non-irrigated corn and concluded that when N rate was increased, soil NO<sub>3</sub> -N was also higher. Another study showed no significant differences in soil NO<sub>3</sub> -N among several N fertilizer rates, although there was a clear trend of higher soil NO<sub>3</sub> -N levels with the highest fertilizer N application which may cause accumulation in the soil profile and leaching into groundwater in the long term (Elmi et

al., 2002). Wang and Alva (1996) observed that up to 30% of N applied as slow release can be leached as compared to more 88% N leaching after readily soluble ammonium nitrate application in sandy soils. Acidic materials alone, organic and inorganic additives, mixture of acidic materials and additives could reduce N loss by 60, 38.5 and 49%, respectively (Zaman et al., 2007). Generally, the main concerns for the above mixtures are that they create an acidic environment from acidic materials used and inhibit ureolytic microorganism's activities which in effect slows down the release of  $\text{NH}_4^+$  into the soil and indirectly reduces N loss (Cheftetz, et al., 1996).

Controlled or slow-release fertilizers can be classified in two basic groups: compounds of low solubility and coated water-soluble fertilizers. Other products, known as N stabilizers or bio-inhibitors, are not true slow-release products, but reduce N losses by slowing N transformations. Polymer-coated controlled-release fertilizers look promising for widespread use in agriculture because they can be designed to release nutrients in a more controlled manner. The polymers are generally durable and exhibit consistent release rates that are predictable when average temperature and moisture conditions can be estimated. Nutrient release rate is altered by manipulating properties of the polymer coating. A more detailed review was provided in (Hauck, 1985). Ureaform fertilizer has the following characteristics: Consists essentially of chemically combined urea with greatly reduced solubility. Nitrogen is released through action of soil microorganisms. Biological reactions are dependent on temperature - require same conditions as growing plants. Quality is indicated by combination of WIN and AI. Other slowly available fertilizers require data such as coating thickness, particle size, soil moisture, and permanganate values to indicate quality. Nitrification studies in soil indicate 30-40% release in 4 weeks, 60-75% in 24 weeks, with a portion being carried over for utilization in the following season. Many researchers stated that nitrogen application increased grain yield and its components of maize. Torbert et al. (2001) found that yield and yield component of maize were increased by increasing the rate of applied nitrogen. El-Sheikh (1998) reported that applying N 160 kg/ha significantly increased ear characters and grain yield of maize. El-Kramany (2001) found that the use of slow release nitrogen fertilizer gave the highest 1000-grain weight, grain yield/plant, grain yield /fed. and nitrogen and protein content of wheat plants compared to the other nitrogen sources. Scott Perin et al. (1998) showed that amending sandy soil with slow release N can reduce N leaching, increase plant growth and increase nitrogen concentration in sweet corn.

Therefore the present research was conducted to evaluate the effect of slow-release nitrogen fertilizers and levels on yield and some nutrients uptake of maize plants.

### **Materials And Methods**

A field experiment was conducted at private farm in located at El-Khatatba, district, Menofia Governorate, Egypt, in summer season of 2009 to evaluate the effect of slow release nitrogen fertilizers at two levels on maize (*Zea mays* L.) (Single – cross 10) grown on new reclaimed sandy soil. Some physical and chemical properties of studied soil are revealing in Table (1).

Table (1): Physical and chemical properties of the studied soil.

Soil properties	Value
Particle size distribution %	
sand	84.00
Silt	10.00
Clay	6.00
Soil Texture	Loamy sandy
Soil pH (saturated soil paste)	8.10
EC(dS/m) in soil paste extract	2.02
CaCO <sub>3</sub> %	8.89

The experiment was conducted in a factorial split plot design with three replicates for each treatment, nitrogen sources (urea, ureaform and urea + humic acid) were in the main plots. N levels were represented the sub plot. Each plot was 3x3.5 m. Calcium super phosphate (15% P<sub>2</sub>O<sub>5</sub>) and potassium sulphate (48%K<sub>2</sub>O) were applied to the all of plots during papering the soil at the rate of 50 and 24 kg fed-1, respectively.

Nitrogen fertilizers treatments as follows: control, 120 kg N fed-1 as urea (46.5%N), 60 kg N fed-1 as ureaform (38%N), 100 kg N fed-1 as ureaform (38%N), 60 kg N fed-1 as urea (46.5% N) + humic acid at rate of 20 kg fed-1 and 100 kg N fed-1 as urea (46% N) + humic acid at rate of 20 kg fed-1. Ureaform was applied at sowing; however urea or urea + humic acid were applied into three equal doses, which applied at 15, 45 and 60 days from sowing.

Plant samples were taken at harvest stage. Plant high (cm), ear weight (g), ear grain weight, shoot and grain yields as well as 100-grain weight were recorded and grains were also subjected to chemical analysis. Whereas, at harvest time (120 days after planting) grain weight kg/plot were measured. Grain yield per feddan was obtained. Total-N in shoots and grains were determined by the kjdhal method described by (Bremner and Mulvaney, 1972). Phosphorus, potassium and micronutrients (Fe, Zn and Mn) were determined according to the method described by Cottenie.

Beneficial effects use of different nitrogen sources and levels on maize growth and N uptake were assessed by calculating following parameters according to Mengel and Kirkby (2001).

- Agronomic efficiency=  $\frac{\text{Yield F} - \text{Yield C}}{\text{Fertilizer N applied}}$

- Apparent N Recovery =  $\frac{\text{N uptake F} - \text{N uptake C}}{\text{Fertilizer N applied}}$

- Physiological Efficiency =  $\frac{\text{Yield F} - \text{Yield C}}{\text{N uptake F} - \text{N uptake C}}$

Were: F= Fertilizer C= Control (without fertilizer) N=Nitrogen

The obtained data were subjected to statistical analyses according to Snedecor and Cochran (1980) using L.S.D. at the level of 5%.

## Results and Discussion

Effect of different N-sources on some growth parameters, shoot, grain and biological yields of maize plant:

Data presented in Table 2 reveal that ear length, plant high (cm), 100-grain weight, shoot, grain and biological yield (ton/fed) of maize plants were significantly affected by increasing levels of different nitrogen sources. It was noticed that the application of different nitrogen sources increased ear length, Plant high (cm), 100-grain weight, shoot, grain and biological yield (ton/fed) of maize plants compared with the control treatment. Results also indicated that the application of ureaform or urea+humic acid increased the abovementioned parameters of maize plants compared with urea as source of nitrogen fertilizer. Also obtained data in

Table 2 show that increasing nitrogen fertilization levels led to a significant increase in all studied traits compared with the control treatment. These increases of the parameters under investigated may be due to the amount of metabolites synthesized by plants as a result of increasing nitrogen levels. This may be attributed to the favorable effect of nitrogen fertilizer levels on the metabolic processes and physiological activates of meristimatic tissues, which are responsible for cell division and elongation in addition to formation of plant organs this lead to more vigorous growth and consequently accumulation of more photosynthesis assimilates. Similar results were reported by (El-Naggar & Amer, 1999; El-Bana & Goomaa, 2000). Generally, the application of ureaform at rate of 100 kg N/fed followed by urea at rate of 100 kg N/fed +humic acid caused the highest values of ear length, plant high (cm), 100-grain weight, shoot, grain and biological yield when compared with other N treatment as urea application at rate of 120kgN/fed. The relative increases were (80.00, 99.05, 30.88, 318.25, 332.22 and 323.78%) and (56.80, 88.78, 23.55, 247.44, 251.11 and 249.78%) for both treatment ureaform at rate of 100 kg N/fed or urea at rate of 100kgN/fed +humic acid, respectively compared to the control treatment. These results may be due to that sandy soil is very low water holding capacity and high nutrient leaching losses. Also application of urea as slow release nitrogen fertilizer or combined of urea with humic acid maintained the nitrogen losses as volatilization or leaching.

Table 2: Effect of different nitrogen sources and levels on ear length (cm), plant high (cm), 100 grains weight (g), shoots, grains and biological yields (ton/fed) of maize plant.

Treatments		Ear length(cm)	Plant high (cm)	100 grains (g)	Shoots ton/fed.	Grain yield (ton/fed.)	Biological yield (ton/fed.)
Sources	Rates of Kg/fed.						
Control	0	11.90	107.00	25.9	1.37	0.90	2.27
Urea	120	17.88	150.00	29.8	3.94	3.11	7.05
Urea+humic acid	60	16.70	139.00	28.0	3.34	2.98	6.32
Urea+humic acid	100	18.66	202.00	32.00	4.76	3.16	7.94
Ureaform	60	15.00	128.00	27.06	3.07	2.56	5.63
Ureaform	100	21.42	213.00	33.9	5.73	3.89	9.62
L.S.D.at,0.05		2.303	3.837	N.S	0.070	0.099	0.090

Hanafi et al. (2002) reported that uncoated compound fertilizer such as urea gave significantly higher amounts of nutrients loss compared to slow release N fertilizer. Concerning the effect of urea + humic acid the obtained results could be due to the urea + humic acid mixtures are that create an acidic environment from acidic materials used and inhibit ureolytic microorganisms activities which in effect slows down the release of  $\text{NH}_4^+$  into the soil and indirectly reduces N loss (Cheftetz et al 1996). El-Kramany (2001) found that slow-release nitrogen fertilizer gave the highest 1000-grain weight, grain and biological yield /fed of wheat. Yerokun (1997) reported that increasing nitrogen supply up to 134 kg N ha<sup>-1</sup> improved maize yield. El-Naggar and Amer (1999) found that maize grain yield was



significantly increases N rate increased and maximum figure was obtained due to addition of 140 kg N/fed.

Effect of different N-sources on macronutrients uptake by shoot and grain of maize plant:

Data in Table (3) revealed that the effect of different nitrogen sources and levels on N, P and K uptake by shoot and grain of maize plant. It is clear from data that application of all nitrogen sources and levels increase N, P and K uptake of shoot, grain and total uptake of their nutrients compared to the control treatment.

Table 3: Effect of different nitrogen sources and rates on N, P and K uptake (kg/fed) by shoot and grain of maize plant

Treatments		Shoot uptake (kg/fed)			Grain uptake (kg/fed)			Total nutrient uptake (kg/fed.)		
Sources	Rates of N Kg/fed.	N	P	K	N	P	K	N	P	K
Control	0	11.76	5.69	16.13	19.15	9.83	11.62	30.81	15.52	27.75
Urea	120	31.23	8.00	30.90	46.21	14.44	19.54	77.44	22.44	50.44
Urea+humic acid	60	24.03	14.92	36.05	38.76	19.21	25.00	62.79	34.13	61.05
Urea+humic acid	100	38.75	18.11	44.67	51.36	26.00	31.08	90.11	44.11	75.75
Ureaform	60	29.45	11.03	38.65	42.90	16.70	23.62	72.25	27.73	62.27
Ureaform	100	43.21	15.54	41.91	67.49	21.56	27.67	110.70	37.10	69.58
L.S.D.at,0.05		2.386	0.907	2.303	5.288	2.301	2.440	2.289	1.819	2.301

The maximum total N uptake of 110.0 kg/fed by maize crop were obtained in treatment (ureaform at rate of 100 kg N/ fed.). While the treatment (urea + humic acid at rate of 100kg N/fed) caused the highest values of both total P and K uptake by maize crop. These results may be due to the use of slow release nitrogen fertilizer or urea combined with humic acid improving of dry matter and grain yield of maize plant and consequently alternative the macronutrients uptake and produced excellent results when compared to urea. Raina and Goswami (1988) stated that the increase in P uptake may be due to the prevention of P fixation in the soil and the formation of humophospho complexes, which are easily assimilable by the plants. Randhawa and Broadbent (1965) reported that HA produces ligands capable of complexing nutrient elements and the complexed elements remain more available to plant roots as complexation shields them against immobilisation in soil. Inhibition of urease activity by HA led to reduced losses of N by volatilization, as described by Flaig (1984) could have also contributed to increased availability of nitrogen. Heng (1989) reported that HA reduces P fixing capacity of the soil, which closely corroborate with the present study where HA enhanced the P availability. The increased N uptake was supposed to be due to the better use efficiency of applied N fertilizers in the presence of humic acid coupled with retarded nitrification process enabling the slow availability of applied N (Guminiski, 1968). Samson and Visser (1989) found that application of humic acid induced increase in permeability of biomembranes for electrolytes accounted for increased uptake of K. Dou and

Alva (1998) conducted a study to measure the growth and N uptake of two citrus rootstock seedlings after application of two controlled-release N fertilizers (polyolefin resin-coated (PRC), sulfur-coated urea (SCU)), and traditional urea. The study demonstrated that the total N uptake by seedlings was greater for the controlled release fertilizers compared to traditional urea.

Effect of different nitrogen sources and levels on Agronomic efficiency, Apparent N recovery and Physiological efficiency:

Figure (1) demonstrated that the effect of different nitrogen sources and levels on Agronomic efficiency, Apparent N recovery and Physiological efficiency of maize plant.

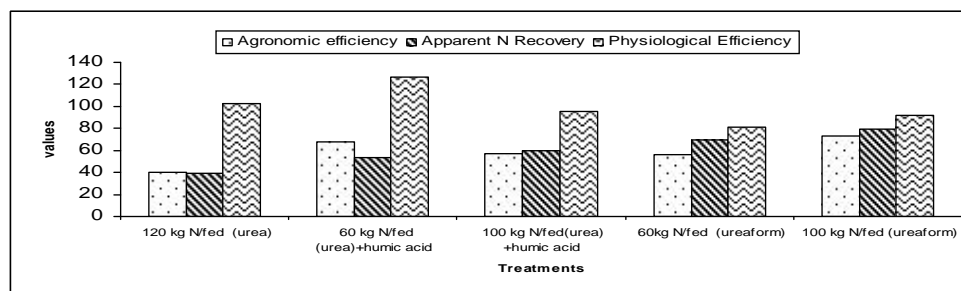


Figure 1. Effect of different treatments on Agronomic efficiency, Apparent N recovery and Physiological efficiency of maize plant.

Results in the Fig 1 suggested that the highest values of Agronomic efficiency and Apparent N recovery could be obtained due to application of (100 kg N/fed as ureaform), while (urea at rate of 60 kg N/fed+humic acid) gave the highest values of Physiological efficiency. This effect may be due to higher utilization of nitrogen by the crop as a result of retardation of losses of fertilizer by the regulation of urea hydrolysis and nitrification and subsequently higher N use efficiency due to regulation of urea-N transformation. Susilawat et al (2009) reported that the amount or rate of humic molecules was enhanced  $\text{NH}_4$  and  $\text{NO}_3$  recovery in soil which can indirectly promote plant growth. Zhu and Chen (2002) suggest for China that emphasis be placed on optimization of N application rates, deep placement of N, matching N application with crop demands, balanced fertilizer application, and use of controlled release N fertilizer of nitrification inhibitors. KANETA et al. (1994) compared coated urea with a conventional compound fertilizer in one single application in a nursery box of non-tillage rice. In his experiment the absorption of N from coated urea was greater than that from the conventional fertilizer (recovery of 79% of N from coated urea at maturity). This also resulted in a greater number of grains and a higher yield. Perrin et al. (1998) showed that amending sandy soils with slow-release N can reduced leaching, increase plant growth and increase N concentration compared with sweet corn grown in soil amended with ammonium nitrate. Amal et al (2007) concluded that Slow-release N fertilizer has long – term effects including reduced leaching losses and enhanced N uptake, as well as positive effects on both health and soil nutrient levels. Therefore amending poor soil with slow-release N fertilizer could be effective in eliminating mid-season N deficiency. (FUJITA, 1996a) showing the possible reduction of the amount of nutrients applied by use of controlled-release fertilizers without affecting the grain yield. Gauer et al. (1992) reports that NUE is generally the greatest with low levels of applied N and decreases as the amount of N applied increases.

### Conclusion

Slow release nitrogen fertilizers can be applied as a preplant application. It reduces production costs, eliminates the need for multiple applications of soluble nitrogen fertilizers. Also slow

release nitrogen fertilizers were able to increase nitrogen use efficiency by reduce nitrogen leashate and volatilization from soils.

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**INVESTIGATION THE EFFECTS OF BIOFERTILIZERS ON VEGETATIVE  
GROWTH PARAMETERS OF MEDICINAL PLANT OF TARRAGON(*ARTEMISIA  
DRACUNCULUS*)**

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**Abstract**

In order to investigate the effects of Plant growth promoting Rhizobacteria (PGPR) inoculation on the morfological traits of Tarragon(*Artemisia dracunculus*) an factorial pot experiment based on completely randomized design with four replication were conducted in Research field of Horticulture Department of Mohaghegh Ardabili University at 2010 - 2011. Experimental treatments include inoculation with three species of rhizobacteria namely *Azotobacter*, *Azospirillum*, *Pseudomonas* suspension in single and combination application and control ( without inoculation with bacterly), which applied as rhizome inoculation and foliar spraying. Result revealed that inoculation of tarragon plants with plant growth promoting Rhizobacteria had significant effect on growth parameters. The highest value for traits such as the number of stem branches and rhizome and leaf number were obtained by foliar application of *Azospirillum* – *Pseudomonas* combination and combined form of three mentioned Rhizobacteria. Rhizome inoculation of *Azotobacter* - *Azospirillum* combination caused increases in plant height and rhizome dry weight in comparison to control. In general results of this investigation indicated that inoculation with plant growth promoting Rhizobacteria leds to increases in growth indices of tarragon plants by enhancing root growth and development by supplying favorable condition for plant growth with respect to supplying better condition for water and nutritional elements absorption from soils.

**Keywords:** Biofertilizer, Plant growth promoting Rhizobacteria, Tarragon, medicinal plant

**Introduction**

Tarragon (*Artemisia dracunculus* L) is one of the medicinal plants belong to Asteraceae family. The origin of this species. is geographically associated with the steppes of Eastern Siberia and Mongolia( ). Tarragon is a perennial plant with a woody rhizome 0.5 – 1.5 cm thick, with a light covering of root hairs, some-times having well-developed underground shoots; the whole plant is bald, smooth, and green, and young plants have only occasional branching. The stalks are straight, single or few in number, 150 cm high, ribbed, more or less branched, the lower branches not bearing flowers. The leaves are unitary, linear or almost linear lanceolate, of size 1.5 – 8.0 cm in length and 1 – 10 (14) mm in width; the lowest sometimes have trifoliolate tips. Flower heads are numerous, spherical, sessile, 2 – 4 mm in width, gathered into clusters at the apexes of the stalk and branches, forming paniculate inflorescences; the bract leaves are smooth, the external ones being elongated almost to the lanceolate, the inner ones being round to oval, wide at the edge and covering the spadix. The marginal florets are pistillate and there are usually seven of them, with tubular corollas widened towards the base; the laminae of the stigma are narrow, linear, and slightly pointed, and extend from the tube divergently. The florets of the disk are staminate and are usually 11 – 14 in number, with conical, quinque-dentate corollas, linear anthers, blunt-angled but slightly pointed terminal appendages, the basal ones being shorter and blunt; the stigma of the

rudimentary pistil is unitary and is funnel-shaped at the apex. The seeds are small, 0.6 mm long, flattish, egg-shaped, finely grooved, and brown. Seed weight is 0.3 – 0.5 g/1000 seeds (Aglarova, 2008).

Plant Growth Promoting Rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield [1]. The mechanisms by which PGPRs promote plant growth are not fully understood but are thought to include: - the ability to produce phytohormones - symbiotic N<sub>2</sub> fixation against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal compounds and also - solubilisation of mineral phosphates and other nutrients (Bashan, 2004; Banchio, 2008; Mahfouz, and Sharaf- Eldin. 2007). The most important plant growth promoting Rhizobacteria which are used in agriculture are Nitrogen – fixing bacteria such as *Azotobacter* and *Azospirillum* and phosphate – solubilizing bacteria belong to *Pseudomonas*. The effect of plant growth promoting Rhizobacteria in facilitation of rooting in mint (*Mentha Piperata*) cutting has been reported (Kamayk, et al 2008), and these increases in root formation is attributed to synthesis of plant growth regulations by PGPRs. Fertilizing of fennel plants with different strains of *Azotobacter chroocum* and *Azospirillum lipoferum* and *Bacillus* and Half doses of NPK decrease production of plant shoots in comparison to single application of nitrogen. They found that these increment of shoot production is related to nitrogen fixation by *Azotobacter* and *Azospirillum* (Mahfouz and Sahar Eldin, 2007). Beaset Mia et al (2010) found that inoculation of in micropropagated seedling of banana with *Azospirillum* leads to increases in Dry weight, length of root and also the number of hairy root, Van loon (2007) reported that increase in lateral root formation in Turfgrass can be related to increases in the level of Auxin by inoculation by pseudoflorescence which may be related to elevation in Auxin synthesis by this Bacteria.

Medicinal plants have an important value in the socio-cultural, spiritual and medicinal use in rural and tribal lives of the developing countries. The main objective of this research was to determine if PGPR strains on growth parameters and grain yield of Tarragon as important medicinal plants.

### **Material and methods**

In order to investigate the effects of PGPRs on Tarragon Factorial pot experiment based on completely randomized design in four replication was conducted in 2010 – 2011 in Research form of Mohaghegh Ardabili university. Experimental treatments include three plant growth promoting bacteria namely *Azotobacter chroococcum* strain 5- *Azospirillum lipoferum* strain of – *Pseudomonas putida*, various combinations of these PGPRs and Control without inoculations.

For instance 95- 100 g of Rhizome were planted in beds containing 15 % v/v of vermicompost. PGPR fertilization carried out as rhizome inoculation and foliar spraying for Rhizome inoculation 40 cc of Diluted suspension from mentioned PGPRs were spread on rhizomes. foliar spraying were done with two month intervals two month after last foliar spraying, plants were harvested and traits such as plant (stem) diameter number of aerial shoots plant fresh and dry weight leaf area, leaf number, Rhizome fresh and dry weights were recorded.

The collected data were analyzed statistically using the Statistical Analysis System (SAS, version 9.0, 2004).

Following the analysis of variance procedure (ANOVA), differences among treatment means were determined using Duncan's New Multiple Range Test (DMRT) comparison method (whenever applicable) at 5% level of significance

## Result

According to means of treatments in tables 1 and 2 inoculation with different plant growth promoting rhizobacteria, have significant effects on tarragon plants growth parameters as follow as :

Plant diameter: As shown in Table 2 inoculation of Tarragon Rhizome with suspension derived from combination of each three PGPR, Azotobacter, Azospirillum and Pseudomonace produced plants with the highest Diameters (39.25 cm<sup>2</sup> ) wich followed by Rhizeme inoculation with combination Azotobacter – pseudomonace and Azetobacter – Azospirillum with means of 33.75 and 31.75 cm respectively. The lowest value for plant diameter(29.25) cm was obtained in Feliar spraying of Azotobacter.

Stem number: The highest number of stems (76.75 and 68 ) were obtained by foliar sraying of combined suspension composed from Azesperillum- pseudomonace and Azotobactere-pseudomonace respectively which have significant Differce with control and other treatments(Table 2).

Rhizome fresh weight : Foliar spraying of azospirillum produceed the highest fresh weight of rhizome (477.25g) which has a significant diferrence with other treatments also Foliar spraying with combination of three plant growth promoting bacteria in this experiment caused poor rhizome growth(205g) (Table 2).

Rizome dry weight: inoculation of tarragon rhizomes with combination of azotobacter and azosperrillum leds to produce 247.5g of dry weight of rhizomes, which highrt than other treatments and combination of three plant growth promoting bacteria caused the lowest rhizome growth (70g) (Table 2).

Rhizome branch number: As shown in table 2 the highest value(31.75) for this trait were obtained by appliction of foliar spraying of azosperrillum- pseudomonas combined suspension which wasn't significant difference with azotobacter and azosperrillum combination (30.5).

Leaf number: The results of comparision of means in table 1 revealed that combined of azosperrillum and pseudomonas pgprs produced the most leaf number in comparision to other treatments.

Plant fresh weight: according to means in table 1 the highest fresh weight of plants were obtained by spraying method of plant growth promoting rhizobacteria and also application of azosperrillum (39.15 g). leaf and total dry weight: inoculation with plant growth promoting rhizobacteria showe similar trend for both of these traits. The highest value for total dry weight (12.731g ) and leaf dryweight(9.73g) were accived by application of azosperrillum genus of pgprs.

## Results and discussion

Results of this experiment revealed that application of these three plant growth promoting rhizobacteria have significant effects on all of traits were studied. And foliar spraying were more efficient in enhancing growth parameters of tarragon plants. Increases in growth of rhizome and plant by these bacteria can be attributed to increases in lateral roots and enhancing in absorption surface and increment of uptake of nutrients in roots. The findings of Abdul-Jaleel et al (2007) van loon (2007) confirms the results of this investigation Azospirillum represents the best characterized genus of plant growth-promoting rhizobacteria. Four aspects of the Azospirillum<sup>^</sup>plant root interaction are highlighted: natural habitat, plant root interaction, nitrogen fixation and biosynthesis of plant growth hormones. Each of these aspects is dealt with in a comparative way. Azospirilla are predominantly surface-colonizing bacteria, whereas *A. diazotrophicus*, *H. seropedicae* and *Azoarcus* sp. are endophytic diazotrophs. The attachment of Azospirillum cells to plant roots occurs in two steps. The

polar flagellum, of which the flagellin was shown to be a glycoprotein, mediates the adsorption step ( Steenhoudt,. and Vanderleyden, 2000)

Table 1. Mean comparison of Inoculation method and pgpr effects on tarragon plants growth

Variables		Leaf number	Total fresh weight	Total dry weight	Leaf dry weight
Control		913.5 <sup>b</sup>	37.406 <sup>a</sup>	7.775 <sup>b</sup>	6.208 <sup>b</sup>
Inoculation method	Rhizome inoculation	1124.69 <sup>a</sup>	39.038 <sup>a</sup>	10.048 <sup>a</sup>	7.852 <sup>a</sup>
	Foliar spraying	1080.63 <sup>a</sup>	35.261 <sup>b</sup>	9.033 <sup>a</sup>	7.177 <sup>a</sup>
Plant growth promoting rhizobacteria	Az	1030 <sup>b</sup>	23.388 <sup>a</sup>	8.195 <sup>b</sup>	6.6350 <sup>b</sup>
	As	1079.1 <sup>b</sup>	36.640 <sup>a</sup>	12.731 <sup>a</sup>	9.738 <sup>a</sup>
	Ps	1085 <sup>b</sup>	30.088 <sup>a</sup>	9.033 <sup>b</sup>	7.376 <sup>b</sup>
	Az- As	1013.5 <sup>b</sup>	28.648 <sup>a</sup>	9.758 <sup>ab</sup>	7.221 <sup>b</sup>
	Az- Ps	1169.1 <sup>b</sup>	29.171 <sup>a</sup>	9.239 <sup>b</sup>	7.340 <sup>b</sup>
	As- Ps	1454.4 <sup>a</sup>	32.107 <sup>a</sup>	10.330 <sup>ab</sup>	8.220 <sup>ab</sup>
	Az-As-Ps	1076.6 <sup>b</sup>	30.604 <sup>a</sup>	9.263 <sup>b</sup>	7.376 <sup>b</sup>

Similar letters in each column indicating non-significant difference at 0.05

Az=Azotobacter As= Azosperillum, Ps= Pseudomonas

Table 2. Interaction effects of Inoculation method and Plant growth promoting Rhizobacterias on tarragon plants growth

Variables		Plant (stand) diameter	Stem number	Rhizome fresh weight	Rhizome dry weight	Rhizome branches number
Control		30 <sup>cd</sup>	36 <sup>d</sup>	208.750 <sup>f</sup>	70 <sup>d</sup>	8.250 <sup>f</sup>
Rhizome inoculation	AZ	35.750 <sup>ab</sup>	51 <sup>bcd</sup>	240 <sup>h</sup>	93.75 <sup>igh</sup>	17.250 <sup>de</sup>
	AS	38.500 <sup>a</sup>	39.500 <sup>cd</sup>	333.75 <sup>d</sup>	140 <sup>c</sup>	20 <sup>c</sup>
	PS	37.500 <sup>a</sup>	42.750 <sup>cd</sup>	381.25 <sup>c</sup>	141.250 <sup>c</sup>	24.500 <sup>b</sup>
	AZ-AS	31.750 <sup>cd</sup>	45 <sup>cd</sup>	416.25 <sup>b</sup>	247.50 <sup>a</sup>	24.750 <sup>b</sup>
	AZ-PS	33.750 <sup>bc</sup>	43 <sup>cd</sup>	277.50 <sup>c</sup>	128.750 <sup>cd</sup>	25.250 <sup>b</sup>
	AS-PS	38 <sup>a</sup>	49.750 <sup>cd</sup>	252.50 <sup>fgh</sup>	112.50 <sup>defg</sup>	25.500 <sup>b</sup>
	AZ-AS-PS	39.250 <sup>a</sup>	40.250 <sup>cd</sup>	268.75 <sup>efg</sup>	116.250 <sup>def</sup>	27 <sup>b</sup>
Foliar spraying	AZ	29.250 <sup>d</sup>	43.250 <sup>cd</sup>	272.50 <sup>efg</sup>	102.50 <sup>fg</sup>	18.250 <sup>cde</sup>
	AS	32 <sup>bcd</sup>	44.500 <sup>cd</sup>	477.25 <sup>a</sup>	195 <sup>b</sup>	20.750 <sup>c</sup>
	PS	32.750 <sup>bcd</sup>	45.500 <sup>cd</sup>	238.75 <sup>h</sup>	99 <sup>fgh</sup>	24.750 <sup>b</sup>
	AZ-AS	30 <sup>cd</sup>	46 <sup>cd</sup>	248.75 <sup>gh</sup>	107.500 <sup>efg</sup>	19.750 <sup>cd</sup>
	AZ-PS	31.750 <sup>cd</sup>	68 <sup>ab</sup>	353.75 <sup>d</sup>	205 <sup>b</sup>	16 <sup>c</sup>
	AS-PS	30.500 <sup>cd</sup>	76.750 <sup>a</sup>	273.75 <sup>ef</sup>	126.250 <sup>ced</sup>	31.750 <sup>a</sup>
	AZ-AS-PS	30.500 <sup>cd</sup>	55.500 <sup>bc</sup>	205 <sup>ij</sup>	78.750 <sup>i</sup>	30.500 <sup>a</sup>

Similar letters in each column indicating non-significant difference at 0.05.

Az=Azotobacter As= Azosperillum, Ps= Pseudomonas

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**THE EFFECTS OF DIFFERENT FERTILIZERS ON  
SPELT GRAIN YIELD (*TRITICUM AESTIVUM* SPP. *SPELTA*)**

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**Summary**

The paper examined the impact of fertilizers in organic farming technology on spelt grain yield (*Triticum aestivum* spp. *spelta*). In mountainous conditions at over 1000 m above sea level research was conducted so as to examine the microbiological effects of different fertilizers (Uniker and Slavol) and soil conditioner (zeolite) on spelt yield in organic farming systems. Organic farming technologies included conventional tillage of soil, where agricultural production has not been organized for ten years. Soil conditioners and fertilizers Uniker microbiological, and combinations thereof are applied by treating the soil just before sowing of spelt (cultivar Nirvana). Microbiological fertilizer (Slavol) was applied in top-dressing.

Applying the statistical analysis of data on grain yield, it was found that the greatest difference in yield was recorded between the investigation years: 4.60 t ha<sup>-1</sup> (2010/11) and 2.82 t ha<sup>-1</sup> (2011/12). When applying top dressing some differences were recorded, but they were not statistically significant (3.66 t ha<sup>-1</sup> : 3.76 t ha<sup>-1</sup>). When it comes to a basic fertilization, the highest grain yield was recorded with a combination of microbiological fertilizers and soil conditioner (4.62 t ha<sup>-1</sup>) and the lowest in the control (2.63 t ha<sup>-1</sup>).

**Key words:** spelt, organic farming, fertilizer, grain yield.

**Introduction**

According to recent literature data spelt was created 7,000 years ago in the area of the Transcaucasus, northern from the Black Sea, most likely in spontaneous crossing of wild grass species. As in other European countries, spelt in Serbia began to grow at the beginning of the last century, especially in mountainous areas. Then for a long time spelt had been neglected as a species, primarily due to the cost of processing (flaking) of its chaff fruits.

Spelt (*Triticum spelta*) is an ancient grain which is related to common bread wheat (*Triticum sativum*) but has certain properties which make it in many respects quite different (Sans *et al.*, 2011). Having fallen from favour as a grain for cultivation in the 19th century following the rapid development in modern farming techniques, spelt is currently enjoying a resurgence in popularity as information about its value as a food source and its ability to be tolerated by many people with wheat sensitivities becomes more widely known.

The quality of grain spelt compared to the soft wheat grain showed increasing in the total protein content (about 19%), and also increasing in content of essential amino acids, B vitamins, oils and mineral salts (Glamo lija *et al.*, 2013). Moreover, it contains more zinc, copper and selenium as well as vitamins A, E and D (Andruszczak *et al.*, 2012). The protein in spelt is such that when the flour is turned into bread it bakes well and results in a very light, soft textured loaf with good keeping qualities which doesn't shed crumbs when sliced (Sans *et al.*, 2011). Spelt is recommended in the children diet, for convalescents and for the sick ones, due to its easy digestibility and healing properties of grain and whole plant in general, which

makes spelt suitable for organic farming. When it comes to organic field production it is necessary to choose species which are not in regular use (alternative) and that's what make them suitable for this type of production (Pearson *et al.*, 2004).

Being low yielding it also thrives without the application of fertilisers even on relatively poor soils. Spelt is also very resistant to frosts and other extreme weather conditions and the grain's exceptionally thick husk protects it from pollutants and insects.

Today, spelt wheat is mainly cultivated in German-speaking countries, but also in Italy, France, and since the 1990s also in the Czech Republic, Hungary and Slovakia. In Serbia interest in this cereal has been gradually growing, therefore more and more often the research is conducted which aims at developing proper agricultural techniques of spelt wheat cultivation under Serbian soil-climatic conditions.

The most suitable regions for spelt growing are those with an altitude of over 800 m, but in the same time in less optimal agro-ecological and soil conditions spelt can also give higher grain yields compared to the soft wheat. Based on the fact of modest requirements in terms of soil and crop management, the aim of this study was to examine the impact of soil conditioner (zeolite), microbial fertilizers (Uniker and Slavol) and their combinations on the yield of spelt grain.

### **Materials and methods**

The following factors were included in investigations: Factor A – Years (2010/11 and 2011/12); Factor B – Top-dressing. In full tillering of spelt we applied 5 l ha<sup>-1</sup> microbial fertilizer "Slavol". Fertilization system is complete with this treatment because it is a natural microbial inoculant that contains two groups of bacteria and nitrogen fixers, as well as some biostimulators. The essence of this preparation consists in the fact that in addition to the supply of plants in nitrogen and phosphorus, and allows production entomotoxins that protect plants from insects. Factor C – Fertilization. The experiment tested four variants of fertilization (factor C): control C<sub>0</sub> without fertilization; C<sub>1</sub>- fertilization with Zeolite (2.5 t ha<sup>-1</sup>), C<sub>2</sub>-fertilization with uniker + zeolite and C<sub>3</sub> fertilization with Uniker (10 l ha<sup>-1</sup>).

The experiments were conducted at the locality of Nova Varoš, village Radijevi i (altitude 1100 m) during 2010/11 and 2011/12 year. Conventional plow tillage was performed at 25 cm depth in mid-September and seedbed preparation was with disc harrows and harrow immediately after plowing. Sowing was done in 01.11.2010 and 20.10. 2011.

As the material served late variety, very resistant to winter conditions (Nirvana), selected at the Institute of Field and Vegetable Crops in Novi Sad. The best results are realized on moderate fertile soils, this variety (with high stem) is sensitive to intensive nitrogen fertilization which can lead to the stem lengthening and than lodging. Yield potential is more than 4 t ha<sup>-1</sup>. The favorable ratio of gliadin and gluten in spelt flour enable preparation of different bakery products with high nutritional value.

After harvest, the yield was measured by elemental plots immediately after threshing and reduced to a moisture level of 14%. All data were subjected to analysis of variance. For individual comparisons, we used the LSD test.

### Meteorological conditions during the period of investigation

Meteorological data on the during vegetation period spelt crops 2010/11-2011/12. are shown in figure1. The data shows better meteorological conditions for winter wheat in initial year of this investigation. In the first year is characterized by large amounts of precipitation in autumn and winter months. Good distribution of precipitation in spring and early summer followed the same time a relatively high average monthly air temperature at all growth stages of winter wheat. The second year of investigation had a bad precipitation distribution in spring period compared with first year. Lack of soil moisture and very high temperature in the second year of this study resulted lower yield of spelt grain.

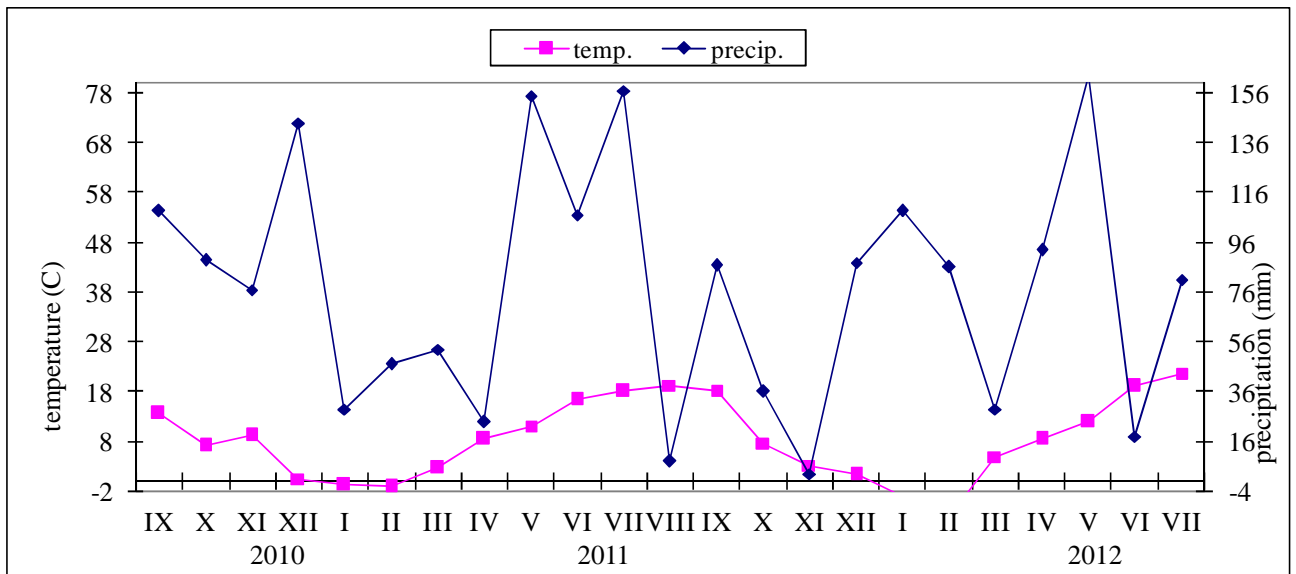


Fig.1. Meteorological data in investigation period on the Zlatibor

### Results and discussion

The results of the effect of organic technology on grain yield of spelt shown in table 1. Based on these results show that grain yield of wheat was higher ( $4.60 \text{ t ha}^{-1}$ ) in first year (factor A) with better meteorological conditions compared with yield in the second year ( $2.82 \text{ t ha}^{-1}$ ). In the dry year of 2011/12 the best results were shown in variants with soil conditioners, as their impact were the greatest in the terms of water insufficiency in the soil. Fertilizers are important factor of organic field production technology alternative small grains (Kova evi *et al.*, 2011) as indicated by our results. The combination of and microbiological fertilizers and soil conditioners gave a significantly higher yield ( $4.45 \text{ t ha}^{-1}$ ) compared with control and variants with only microbiological fertilizers ( $2.61$  and  $3.05 \text{ t ha}^{-1}$ ). Interestingly, the differences between the two type of fertilization more pronounced in favorable years for winter wheat. In the less favorable meteorological conditions due to lack of moisture is missing the full effect of both type fertilizing. If we compare the interaction between of two AB factors (years x top-dressing) can be seen that all interactions in the first year of study had a significantly higher yields than the same interaction in the second years. Yield results in the interaction AC (years x fertilization) shows the same tendency as in the previous case.

The greater effect of fertilization was achieved in years with favorable meteorological conditions. In wheat growing areas with low rainfall levels and high air temperatures, better

results in spelt wheat cultivation are obtained when no-till is used, while plough tillage yields better results in areas with higher rainfall levels (Wo niak, 2013).

Under conditions of the conducted experiment, spelt wheat yielded on the level from 2.61 to 4.54 t ha<sup>-1</sup> (without top-dressing) and from 2.65 to 4.81 t ha<sup>-1</sup> (with top-dressing) and Andruszczak et al., (2011), stated interval grain yields of spelt from 4.07 to 4.45 t ha<sup>-1</sup>, independent of the level of mineral fertilization and chemical protection. Furthermore, according to the same author's application of increased rates of mineral fertilizers increased the grain yield and number of ear-bearing culms per 1 m<sup>-2</sup>, however it had no effect on the ear length, number and weight of grains per ear and the weight of one thousand grains.

Tab. 1. The effect of technology based on organic principles on grain yield of spelt (*Triticum aestivum* ssp. *spelta*) (t ha<sup>-1</sup>)

Years (A)	Fertilizers (C) and Top-dressing (B)								Average (A)
	Control		Zeolite		Uniker+Zeolite		Uniker		
	B <sub>0</sub>	B <sub>1</sub>	B <sub>0</sub>	B <sub>1</sub>	B <sub>0</sub>	B <sub>1</sub>	B <sub>0</sub>	B <sub>1</sub>	
2010/11	3.00	3.11	5.78	5.90	5.53	6.15	3.73	3.63	<b>4.60</b>
2011/12	2.21	2.20	3.29	3.38	3.37	3.46	2.37	2.27	<b>2.82</b>
Average (AB)	<b>2.61</b>	<b>2.65</b>	<b>4.54</b>	<b>4.64</b>	<b>4.45</b>	<b>4.81</b>	<b>3.05</b>	<b>2.95</b>	<b>3.71</b>

B<sub>0</sub>-without top dressing (Slavol); B<sub>1</sub>- with top-dressing (Slavol)

LSD	A	B	C	AB	AC	BC	ABC
0.05	0.10	0.10	0.14	0.14	0.20	0.20	0.29
0.01	0.18	0.18	0.25	0.25	0.35	0.35	0.49

Dolijanovi *et al.*, 2012 stated that the highest yields of grain spelt were obtained by a combination of organic and microbial fertilizers (5.84 t ha<sup>-1</sup>), a microbiological (4.34 t ha<sup>-1</sup>) and in the case of the control treatment without fertilizer (3.86 t ha<sup>-1</sup>). In the dry year, yields were significantly lower, primarily due to the lack of full effects of the applied fertilizers.

Various alternative species of winter wheat such as (*Triticum durum*, *Triticum spelta* and *Triticum aestivum* ssp. *compactum*) can be very interesting for manufacturers because they are selected for specific purposes (Kova evi *et al.*, 2007a). In the case of grain yield of above mentioned alternative species of winter wheat, in research of Kova evi *et al.*, (2007b), the highest yield had *Triticum spelta*, which is species with modest requirements in soil condition and crop management as well as the specific purpose and quality.

## Conclusions

According to the obtained results during two year investigations of effects four fertilization (3 and control) and top-dressing under organic farming practice, the following conclusions can be made:

The results of our investigation show that yield grain of wheat was higher in first year with better meteorological conditions. The effect of applied microbiological fertilizers and soil conditioners mainly was dependent of the amount and distribution of precipitation, especially during the growing season of crops.

Organic field crop technology that includes a combination microbial fertilizer in basic fertilization and top-dressing, with conditioner of soil in recharge gives the highest yield.

Spelt recharged with microbial formulation (Slavol) gave higher yields compared to the variant where was used only the basic fertilization. This results could be usefull for organic growers.

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## VEGARD – BOTANICAL FUNGICIDE

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### Abstract

Powdery mildew, caused by the fungus *Sphaerotheca pannosa* var. *rosae*, appears as a white powdery growth on rose leaves, stems, buds, or flowers.. We studied the development of *S. pannosa* var. *rosae* in roses in Serbia and protection with Vegard 0.5% AS. The trials were set according to the instructions of methods PP1/152(2) (EPPO, 1997) and PP1/104(2). Phytotoxicity was estimated by PP 1/135(2) (EPPO, 1997). The differences of the disease intensity were evaluated by the analysis of variance and LSD-test. The results of the research have demonstrated that there is no statistically significant difference between mid-treatments of other variances and all other treatments, and the differences are incidental. The summary of meteorological data and intensity of infection during the experiment are controlled.

**Key words:** Vegard, *Sphaerotheca pannosa* var. *rosae*, roses, efficacy

### Introduction

Powdery mildew affects more the 7600 species of hosts worldwide, including subsistence crop (Ridout, 2009). They affect virtually all kinds of plants: cereals and grasses, vegetables, ornamentals, weeds, shrubs, fruit trees, and broad-leaved shade and forest trees.

Rose powdery mildew is caused by the fungi *Sphaerotheca pannosa* var. *rosae*, a member of the *Ascomycete* fungi. It infects a wide variety of roses, but especially those grown in dryer climates (Gubler, 2011). Under conditions that are favorable for disease development, powdery mildew can cause complete defoliation. It appears as a white powdery growth on rose leaves, stems, buds, or flowers.

Effective management of rose powdery mildew begins by using resistant varieties of rose, but it can also be managed through the use of fungicides, or by planting in sun since rose powdery mildew prefers the shade (Gubler, 2011). In fact, increasing the exposure of rose powdery mildew from 18 to 24 hours of light per day reduced the production of conidia, the asexual spores of the fungi, by as much as 62% (Suthaparan, 2010). There are a variety of fungicides that have proven to be effective. Examples are mylobutanil, azoxystrobin, triadimefon, and thiophanate-methyl formulations (Gubler, 2011). Chemical fungicides are not always necessary, however, it is possible to use more environmentally-friendly solutions such as a water-vinegar spray, or mixtures of baking soda and insecticidal soaps (Mouchet, 2003). Recent studies have also shown that using a planting medium which includes silicon can also reduce the occurrence of powdery mildew by as much as 57% (Datnoff, 2006).

There are many fungicides registered for control of powdery mildew on roses. In 1998 we lost use of triforine which was especially effective for control of powdery mildew as well as rust and black spot. Some of the most effective products are found in the sterol inhibitors (triazoles and imidazoles) including propiconazole, myclobutanil, triflumizole and triadimefon. Others are found in the strobilurin group including azoxystrobin, kresoxim

methyl and trifloxystrobin. The products listed fall into 7 distinct chemical groups and rotation between groups is highly recommended. Care should be taken to test all new products for safety on your rose cultivars. Some of the "environmentally friendly" products are not always safe for our ornamental crops.

Increasing public concern over the use of chemical pesticides has made the development of biological control agents for powdery mildew highly desirable, since they could provide an alternative to or reduce dependency on currently used control measures. Moreover, the emergence of fungicide-insensitive variants of *Sphaeroteca fuliginea* (Schlectend:Fr.)Pollacci, the fungus responsible for powdery mildew on cucumber has been reported (McGrath, 1991, Schepers, 1983).

The biggest problem with the chemical are and the induction of pathogen resistance. Biofungicides have more specific effects on the target body from chemicals, therefore only affect the target organism and not cause toxigenic effects on the environment and thereby increase biodiversity and wildlife. Development of new, harmless sustainable strategies - biopesticides which involves the use of beneficial micro-organisms or their metabolic products (as an alternative to chemical, synthetic compounds) is conditioned by the demands of the global need for reduced use of chemical pesticides that are considered harmful.

There is accumulating evidence that several biological control agents can be effective antagonists of powdery mildew. *Ampelomyces quisqualis* Ces. (Sundheim, 1982, Rajkovic, 2010), *Acremonium alternatum* Linc:Fr. (Malathrakis, 1985), *Tilletiopsis* spp. (Hijwegen, 1986, Klecan et al., 1990), and *Sporothrixflocculosa* Traquair, Shaw and Jarvis (Belanger et al., 1994, Hajlaoui and Belanger, 1993) have been reported to attack mycelia and reproductive structures of mildew fungi.

Given an increasing international demand to reduce the use of toxic pesticides, because of human health and environmental concerns (Tjosvold & Koike 2001), there is a need to seek more benign disease control alternatives. This is particularly relevant to the powdery mildew /rose interaction, since powdery mildew control can account for up to 40% of the pesticide volume applied to rose crops (Tjosvold & Koike 2001).

Milk has been reported to be effective for the control of powdery mildew in squash and wine grape crops (Bettiol 1999; Crisp & Bruer 2001), but the use of raw milk has had problems including difficulties in handling/storage and unwanted growth of nontarget organisms. Different fractions of milk were tested and AMF was found to be the most toxic to powdery mildew on glasshouse-grown squash (K.V. Wurms, unpublished data). The anti-fungal activity of plant oils against powdery mildew in tomato has also been reported (Ko et al. 2003).

The purpose of this study was to evaluate emulsified formulations of Vegard 15% AS to control powdery mildew in glasshouse-grown roses and to establish if there were any plant health issues associated with regular applications. This represents a novel use for these products.

### **Material and methods**

The studies were performed on the Roses (*Quercus robur* L.) varieties Candid profit in the locality Gložan, old 5 years, growing on the bush form, with the distance of planting 1.5 x0.5, the phenological stages before flowering.

The trials were set in accordance with methods PP 1/152 (2) (EPPO, 1997) and the treatment plan was made according to fully randomized block design. The experiment was conducted on species of roses Candid in four repetitions on basic plots consisting of 8 roses (1,5x0.5 m apart), 25 m<sup>2</sup> in total.

The estimation of leaves with secondary infection with powdery mildew was conducted as follows: on 10 plants, on 50 leaves. The scale of values which was used to record



the results of each leaf is as follows: 0 = no infection, 1 = very low infection, 2 = partial attack (scattered spots affected by powdery mildew), 3 = moderate to severe disease (up to half of the leaf surface is affected by powdery mildews), 4 = very severe disease (more than half of the leaf surface under the powdery mildew; edges of the leaves begin to crumple and dry up).

Regarding the method of application and amount of water per unit surface, the fungicides were applied using the backstroke sprayer “Solo”; with the consumption of 1000 l/ha of water.

The biofungicide was applied on: I 1.06.2012. – Phenophasis-developed shoots, II 18.06.2012. – Phenophasis-developed shoots and bud, III 25.06.2012. – Phenophasis - beginning of flowering.

Biofungicide Vegard 0.5% AS is a highly bioactive plant-derived (*Rheum officinale* Baill) fungicide newly developed by Beijing Kongbo Biotech Co., Ltd. The fungicide provides excellent preventive effectiveness on powdery mildew of vegetables, as well as gray mold and anthracnose. It is very low toxic to human being and livestock and friendly to environment.

Fungicide Benomyl - Active ingredient: Benomyl, Declared: 50.00%, found:  $50.38 \pm 0.20\%$ , method (CIPAC Handbook, 1988, D, 14), number of repetitions: 4, Standard deviation is calculated according to Murray and Spiegel (1961)

The appearance and development of powdery mildews is followed with the initial appearance and development of the disease on the control variation, as well as through accomplishment of a clear difference between the control and other variations on which biofungicides were applied.

The intensity of disease was assessed by the method of EPPO: Guideline for the efficacy evaluation of fungicides - *Sphaerotheca pannosa* var. *rosae*, no. PP 1/104(2) (OEPP, 1997 d). in Guideline for the efficacy evaluation of Plant Protection Products, 1997, 100-102. Time of estimation was 11/07/2012. Phytotoxicity was estimated according to instructions of PP methods (1/135 (2) (OEPP, 1997).

Data processing was performed using standard statistical methods (intensity of infection according to Townsend-Heuberger, the efficiency according to Abbott, analysis of variance according to Duncan test and methods PP/181 (2) (EPPO 1997). The differences of the disease intensity were evaluated by the analysis of variance and LSD-test.

### Results and discussion

In the Table 1 we are presented data on the intensity of attack of *Sphaerotheca pannosa* var. *rosae* on the leaves of roses in the locality Gložan. Tested fungicides had shown statistically significant efficacy compared to the control, in which the intensity of the disease was 31.12%.

Table 1: The intensity of the attacks *Sphaerotheca pannosa* var. *rosae* on the leaves of roses and efficacy of fungicides and biofungicides

No	Fungicide	Conc.( %)	Infection(%)	Efficacy (%)	Standard (Benomyl =100%)
1	Vegard 0.5%AS	4,0	0.50 a	98.38	100.34
2	Benomyl WP 50	0,04	0.61 a	98.04	100.00
3	Untreated	-	31.12 b	0.00	0,00

Lsd<sub>0.05</sub>

11.47

In the locality Gložan is determined the intensity of infection of 31.12% on control variant. On this infection examined preparation showed satisfactory efficacy at the recommended concentration (98.38%). There are no significant differences between the tested fungicides and standard products whose efficiency is 98.04%.

Based on the variance analysis of the randomized block design it was determined that the difference between the mid repetitions was statistically significant at the probability of 95%, since  $F_0 > F_{0,05}$ . Moreover, a statistically significant difference wasn't found between mid treatments at the probability of 99%, since  $F_0 > F_{0,01}$ . Between the mean values of control and variance Vagard0.5%AS there is no a statistically significant difference at the probability of 99%.

By means of a multiple comparison procedure (Duncan test, 1955) one homogenous groups were identified with statistically significant differences at 99%, which match the previously explained groups studied in the variance analysis.

Vagard 0.5% AS are new biofungicide and there are no other similar trials with him. This is preliminary trials in this field.

### Conclusion

Biofungicide Vagard 0.5% AS at a concentration 4% use very effectively against *Sphaerotheca pannosa* var. *rosae* (98.38%) and can be successfully used to protect roses from the causal agent of powdery mildew of rose. It has good characteristics and physical-chemical properties making it suitable for practical application.

Biofungicide Vagard 0.5% AS have more specific effects on the target body from chemicals, therefore only affect the target organism and not cause toxic effects on the environment and thereby increase biodiversity and wildlife.

Using biofungicides in powdery mildew controlling we reduce the induction of pathogen resistance.

### Acknowledgements

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**DEHYDRINS FROM ARABIDOPSIS THALIANA EXPRESSED IN E. COLI  
PROTECT MEMBRANES DURING FREEZING**

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**Abstract**

As the name dehydrins implies, these proteins are typically expressed in response to dehydration which can be caused by drought, osmotic stress or freezing temperatures. In general, dehydrins occur in plants as multi-gene families. *Arabidopsis* dehydrins (LTI29, ERD14, COR47 and RAB18) have been tested for protection of thylakoid membranes during freeze thaw cycle *in vitro*. The results obtained in this study show that dehydrins LTI29, ERD14, COR47 protect thylakoid membranes at low temperatures. A correlation between level of cryoprotective activity and protein concentration has been highlighted. The preliminary results of this study indicate a possible mechanism of cryoprotection in plants.

**Keywords:** *Arabidopsis thaliana*; dehydrins; freezing tolerance; thylakoid; cold acclimation.

**Introduction**

Acclimation capacity of plants

Plants differ in their capacity to cope with sub-optimal temperatures. Chilling sensitive plants, often growing in tropical areas, are injured at temperatures just below +10 °C. Chilling tolerant plants can tolerate low, non-freezing temperatures, but are killed in temperatures a few degrees below zero. Plants that can tolerate freezing temperatures employ two major strategies. They either avoid freezing or tolerate extracellular freezing (Sakai and Larcher, 1987).

Dehydrins (main characteristics)

Dehydration is a common process during seed maturation, which is realized by programmed expression of specific genes. The phytohormone abscisic acid (ABA) plays a key role in the regulation of this process. Several genes specifically expressed in this ontogenetic period have been recognized. They include genes encoding LEA (Late Embryogenesis Abundant) proteins. LEA proteins have been found in ABA treated vegetating plants and also under the stress conditions that result in cellular dehydration induced by drought, salinity, or low temperatures. One group of such genes encodes dehydrins (DHN), known also as group 2 late embryogenesis abundant (LEA) proteins. Nowadays, genes encoding DHNs have been cloned from numerous plant species belonging to such diverse groups as angiosperms, gymnosperms, mosses and lycophods (Svensson et al., 2002).

### Structure of *Arabidopsis thaliana* dehydrins

(RAB18, LTI29, LTI30, and COR47). Isolation and purification of native *Arabidopsis* dehydrins (Svensson et al., 2000) allowed the investigation of their biochemical properties *in vitro*. Generally, *Arabidopsis* dehydrins are enriched with glycine and lysine residues, but they lack cysteine and tryptophan (Wisniewski et al., 1999).

### Cryoprotective activity of Dehydrins

Many studies reported a positive correlation between the accumulation of dehydrin transcripts or proteins and the tolerance to freezing, drought and salinity (Rodriguez et al., 2005; Nylander et al., 2001; Houde et al., 1992).

Puhakainen *et al.* (2004) provided the data that overexpression of multiple *Arabidopsis Dhn* genes such as *LTI29* (*ERD10*, SK3-type) and *LTI30* (K6) resulted in increased freezing tolerance and improved survival under exposure to low temperatures, demonstrating that dehydrins contribute to freezing tolerance. In another side overexpression or antisense inhibition of the RAB18 (*Y2SK2*) gene had no effect on freezing tolerance in *Arabidopsis* (Lang and Palva, 1992).

## Materials and methods

### Plant sources utilized in this study

Spinach (*Spinacia oleracea* L. cv Monnopa) was grown under non-hardening conditions in a growth chamber with 12 h of light at 150 pmol quanta m<sup>-2</sup>sP1 at 25 °C and 12 h of dark at 15°C at 50% RH. (Production: Julius Wagner GmbH)

Cabbage (*Brassica oleracea* L. cv Grüfiwi) was grown in the garden for several months and then transferred to pots. Plants were harvested, and leaves were either used directly for protein extraction or were stored frozen at -20°C.

### Bacterial strain

*E. coli* M15[pREP4], SG13009[pREP4] Qiagen was used for regulated high-level expression with pQE Vectors (Cells contain pREP4 plasmid encoding lac repressor in trans, ensuring tightly regulated expression).

### Expression vector

Analyzed proteins have been expressed using the vector pJTS1. This vector was modified pQE-60 vector by Jan Svensson (Sweden, Uppsala University) (Svensson, 2000).

### Colony screening by PCR

PCR is used to amplify specific regions of a DNA strand. The set of primers used in this study are listed in Tab.1.

### Tab. 1. Lists of oligonucleotides (primers) used in this study

**PQE F1** 5'CCCGAAAAGTGCCACCTG3'

**PQE F2** 5'CGGATAACAATTTACACAG3'

**PQE R** 3'GGTCATTACTGGAGTCTTG5'

LTI29 5'GAAAAGAATGGCAGAAGAGTACAAGAACACC3'

LTI29 3'TTAATCAGACACTTTTTCTTTCTTCTCT5'

ERD14 5'CCGCTCGAGAAAAGAATGGCTGAGGAAATCAAGAATG3'

ERD14 3'GCTCTAGATTATTCTTTATCTTTCTTCTCC5'  
COR47 5'GAAAAGAATGGCTGAGGAGTACAAGAACAACG3'  
COR47 3'TTAATCATCAGACTCTTTTTCTTTCTTCACTTCC5'  
RAB185'CCGCTCGAGAAAAGAATGGCGTCTTACCAGAACCGTCCGTCCAGG3'  
RAB183'GCTCTAGATTAACGGCCACCACCGGGAAGCTTTTCC5'

#### Protein expression

The optimal OD value depends on the method and the medium. For flask cultures using LB-medium an **OD<sub>600</sub>** of **0.6** is recommended.

#### Protein extraction from *E. coli*

Cells were lysed by a *Lysozyme treatment plus sonication* as follows: add lysozyme to a final concentration of 100 µg/ml from a freshly prepared 10 mg/ml stock in water. Incubate at 30°C for 15 min. Mix by swirling and sonicate on ice using a microtip with the power level set between 4–5. Sonicate 4 times for 45 sec. Incubate at 90°C for 8 min. Take a 1.5 ml sample of the lysate and centrifuge at 14,000 g for 10 min to separate the soluble and insoluble fractions. Transfer 100 µl of the soluble supernatant to a new tube. Add 100 µl of 2X Sample Buffer (2X SB = 100 mM DTT, 2% SDS, 80 mM Tris-HCl, pH 6.8, 0.006% bromophenol blue, 15% glycerol) to 100 µl soluble fraction sample. Store at –20°C until SDS-PAGE analysis. Supernatant and solubilised cell debris were analyzed for the presence of inclusion bodies.

#### Cryoprotective leaf proteins extraction and Freezing test

The extraction of leaf proteins and the freezing test were carried out by following the procedures described by Hinch and Schmitt (1992)

##### *Freezing test*

All centrifugation steps take place at 4 °C.

1. *Thylakoid isolation*: 50 g of spinach leaves was homogenized with 100 ml of homogenization buffer in a blender for approx 10 s. 125 µl of 1 M Na-ascorbate and 340 µl of 1M cysteine was added to the buffer immediately before use, as these substances are unstable in solution. Centrifugation at 7000 g for 5 min was done with the aim of washing thylakoid pellet; this procedure was repeated twice. Pellets from the last centrifugation step were resuspended in a minimum volume of washing solution. 10 µl of the thylakoid suspension was mixed with 990 µl of 80 % (v/v) acetone and centrifuged for 2 min in a benchtop centrifuge. Absorbance of the supernatant at 663 and 645 nm with 80 % (v/v) acetone as the reference was measured. Chlorophyll content is calculated as follows:

Chlorophyll content

2.  $(8.02 * A_{663} + 20.2 * A_{645}) * 0.1 = \text{mg Chlorophyll / ml}$ . Hinch and Schmitt (1992)

Thylakoid suspension was diluted with washing buffer to a concentration of at least 1 mg chlorophyll/ml.

3. 0.5 ml of the thylakoid suspension was mixed with an equal volume of proteins suspension in Eppendorf tubes.
4. The suspension was placed in a freezer at -20 °C for 2 h.
5. Samples are most conveniently thawed in a water bath at room temperature and should be transferred to an ice bath immediately when the ice in the tubes has melted.

#### Thylakoid Volume Measurements

Aliquots of thylakoids in cryopreservation solution were mixed with sucrose solutions ranging in concentration from 20 to 500 mM sucrose finally. All sucrose solutions were made in 5 mM MgCl<sub>2</sub>.

The osmolality of the resulting solutions was measured with an osmometer. These measurements are made easier by using the cryopreservation solution diluted 1:1 with the washing solution instead of thylakoids. The final results will be the same.

Hematocrit capillaries were loaded with the diluted thylakoid suspensions and the capillaries were sealed at one end. Then they were centrifuged for 15 min in a hematocrit centrifuge and pellet heights were measured with a magnifying glass and a 0.1 mm scale.

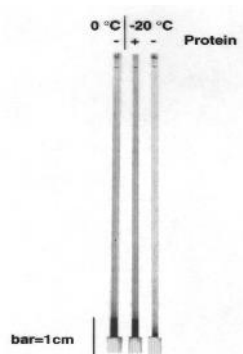


Fig.1. Volumetric test – Hematocrit capillaries were used to measure cryoprotective activity. Controls at 0 °C and -20 °C show whole volume of thylakoids at these temperatures after centrifugation.

The cryoprotective activity (in percentage) was calculated as follows:

$$\text{TKV(PP -20°C)} - \text{TKV(-20°C)} / \text{TKV(0°C)} - \text{TKV(-20°C)} = \text{X}/100$$

TKV(PP -20°C) - thylakoid volume in presence of analyzed protein at -20°C

TKV(-20°C) - thylakoid volume without protein at -20°C

TKV(0°C) - thylakoid volume without protein at 0°C

### Results and discussion

Transformation and checking by colony PCR



*E. coli* M15 strain was transformed. Screening for positive clones was performed with PCR using constructed primers for each gene individually.

Previously the PCR program was standardized (suitable annealing temperature) for all analyzed genes. 94 °C for 60 sec, 94 °C for 30 sec, \* °C for 30 sec 35 cycles, 72 °C for 1 min.

Annealing temperature: (\*) LTI29 56 °C; ERD14 50 °C; COR47 63 °C; RAB18 63 °C

PCR products were analysed by agarose gel electrophoresis (Fig. 2).

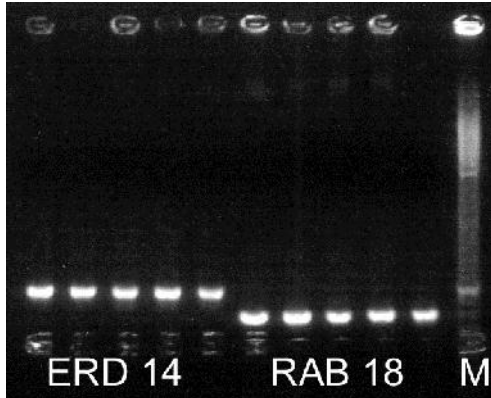


Fig. 2. PCR products for ERD 14 and RAB 18 genes respectively

#### Dehydrins expression

Maximal production of soluble dehydrins was obtained by inducing expression of the cloned dehydrin genes with IPTG when the cells reached an  $OD_{600}$  of 0.5-0.7. Recombinant proteins were localized in the supernatant and did not form inclusion bodies. For heat fractionation, lysates were placed in a 90 °C water bath for 8 min. Approximately 80% of the contaminating proteins precipitated during the heat fractionation. Using concentration filters final concentration was doubled. Cryoprotective dehydrins were analyzed in SDS – PAGE after purification and concentration (Fig. 3).

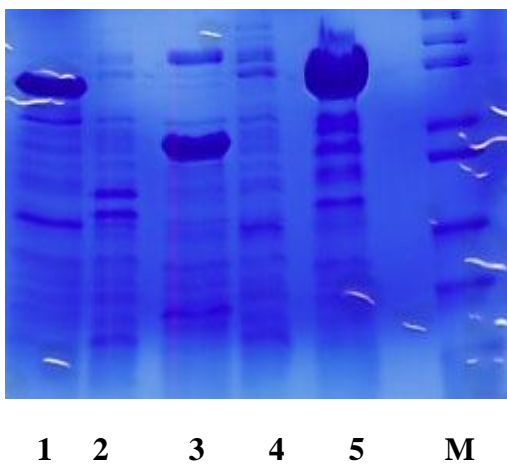


Fig. 3. SDS - PAGE analyses of three cryoprotective dehydrins (LTI29, ERD14 and COR47) after purification and concentration. From gel is visible relation between expression yields of analysed proteins. 1 – LTI29 induced; 2-LTI29 no induced; 3-ERD14 induced; 4-ERD14 no induced; 5-COR47 induced; M-protein marker

#### Protein desalting

The graph in Fig. 4 was constructed for two analysed proteins LTI 29 and RAB 18.

It was shown that protein LTI 29 flow through column after 1,5 ml eluting buffer and the highest concentration of protein was reached at 2,5 – 3,5 ml. The similar elution kinetic was found for other dehydrins except RAB 18. Elution kinetics for protein RAB 18 is different because of smaller size of this protein.

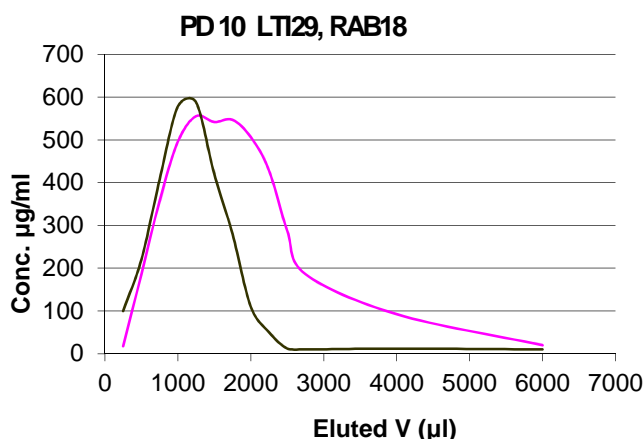


Fig. 4. Protein desalting using desalting columns PD 10. Using desalting columns ammonium sulphate was removed from solution containing analysed proteins. It was shown that proteins LTI 29 and RAB 18 have different elution kinetics because of different dimensions of analysed proteins.

#### Cryoprotective activity

In previous reports it was shown that dehydrins are expressed during the period of plant acclimatization to low temperatures, which points to possible cryoprotective activity of these proteins (Thomashow, 1990). In this study a freezing test based on measuring the ability of proteins to protect thylakoids during a freeze-thaw cycle was used. The results showed that three of four analyzed dehydrins had cryoprotective activity (Fig. 5). In addition to these results dehydrin RAB18 had a low cryoprotective activity (in %) over the level of the negative control. As negative control non induced crude bacterial extracts were used, which showed no cryoprotective activity. As positive control a CPP was used (Hincha et al., 1996). The test confirmed that analyzed dehydrins (LTI29, ERD14, COR47) from *Arabidopsis thaliana* have cryoprotective activity.

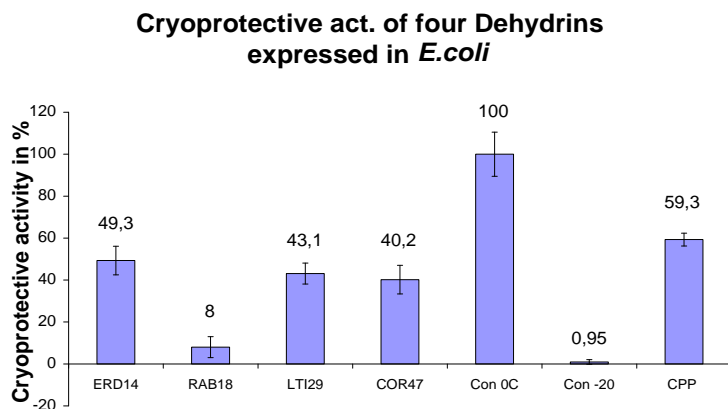


Fig. 5. Cryoprotective activity for four analyzed dehydrins expressed in %.

The results of “freezing test” showed that four dehydrins had different cryoprotective activity. It also revealed that dehydrin RAB18 had low cryoprotective activity. A crude extract of cold hardened *Brassica oleracea* containing cryoprotectin (Hincha et al., 1996) was used as a positive control (CPP).

The concentration of proteins is a relevant factor for the level of cryoprotective activity. During increasing of the initial protein concentration, the cryoprotective activity also increased. Saturation was reached at 50% - 60% activity.

### Conclusions

The cryoprotective activity was proven for 3 of 4 analyzed recombinant dehydrin proteins (LTI29, COR47 and ERD14) expressed in *E. coli* by using freezing test. For the protein RAB 18 a low cryoprotective activity has been shown in this test. Due to the process of heat treatment most of the proteins from bacterial supernatant become denaturalized, while the dehydrins remain heat stable. This was shown to be an important step in the purification of dehydrins. Also the yield of proteins reached satisfactory level although some differences in the yield among the analyzed proteins were noticed. Cryoprotective activity of dehydrins is in positive correlation to the concentration of the analysed proteins used in the assay.

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**THE PHYSICO-CHEMICAL PROPERTIES AND FATTY ACID COMPOSITION OF THREE DIFFERENT HAZELNUT VARIETIES COLLECTED AT THE DIFFERENT HARVEST PERIODS**

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**Abstract**

The objective of current study was to determine the chemical properties of hazelnuts collected at the different harvest periods from Giresun province in Turkey. The oil contents of the first harvest period ranged from 12.3% to 6.51%. The moisture contents of nuts were found low in the same period. The oil contents of hazelnuts harvested at the last harvest period were ranged between 53.40% (sharp) to 66.11 (black). In general, palmitic, stearic, oleic, linoleic acid were identified as dominant fatty acids. Depending on the cultivar and harvest, the oleic acid have been identified at the highest rate and have been partial differences among the varieties. The oleic acid contents of varieties were determined between 74.79% to 85.58% depending on harvest period. Linoleic acid content was ranged from 5.70 to 15.64 %, palmitic acid content ranged from 4.92% to 7.31%. As other fatty acids was found at the minor level. The highest palmitic, oleic and linoleic acid contents have been identified respectively in Tonbul (II.harvest), Black (II.harvest) and Tonbul (I.harvest) varieties. The optimum harvesting time as depending on the physico-chemical properties of the all hazelnut varieties is understood to be August and September month.

**Key words:** hazelnut, varieties, harvest periods, proximate, fatty acid composition

**Introduction**

Hazelnut tree, *Corylus avellana* L., is distributed widely in North of Turkey. It is a shrub or tree from Betulaceae family, Corylaceae family and Corylus genus (Bonvehi and Coll, 1993; Parcerisa *et al.*, 1995; Özdemir *et al.*, 2001; Alasalvar *et al.*, 2003). Hazelnut is among the first species which migrated to northern Europe in the ice age in 17 to 18 thousand BC In seven thousand and five hundred years to eight thousand BC hazelnut had a wide habitat in Europe. However, its habitat has been decreased since 5500 BC due to human activities. The oldest existing knowledge on hazelnuts dates back to the year 2838 BC (Darabzand, 2000). There are several cultivars with various botanical features and different economical values. It is, therefore, important to determine their nutritional properties, and to establish their lipids to find out if the results agree with those reported in literature for hazelnut of other origins (Diaz *et al.*, 1980; Bonvehi and Coll, 1993; Parcerisa *et al.*, 1995; Özdemir *et al.*, 2001; Alasalvar *et al.*, 2003). Turkey is the main hazelnut producer in the world. Turkey's total export revenue from hazelnut and hazelnut products is approximately 1 billion U.S. dollars annually (Turkish Hazelnut Union; Alasalvar *et al.*, 2003). Among the nuts, hazelnut has an important role in human nutrition and health, because of its special fatty acid composition, which includes oleic and linoleic acids as well as the presence of tocopherols and sterols. Also, it provides an excellent source of energy due to its high oil content of approximately 60% (Parcerisa *et al.*, 1995; Amaral *et al.*, 2003; Alasalvar *et al.*, 2003). It is rich in protein and oil (Ba *et al.*,

1986). Recently, a great deal of attention has been given to the hazelnut and their oil, and their consumption has thus increased, especially in Europe countries. In this study, hazelnuts growing in middle Anatolia were used firstly. The aim of this study was to investigate the chemical properties of hazelnuts collected at the different harvest periods from Giresun province in Turkey.

### Material and methods

Hazelnut varieties (Kara, Tonbul and Sivri) were collected by hand in different harvest periods in Giresun, Turkey in 2012. Fruits were cleaned in an air condition, and then stored in polypropylene bags at room temperature. Each sample was analysed as the whole nut, without the shell. Physical and chemical properties of hazelnut fruits were analysed according to Matthaus and Özcan (2012). The oil content was determined according to the method ISO 659:1998 (ISO,1998). About 2 g of the kernels were ground in a ball mill and extracted with petroleum ether in a Twisselmann apparatus for 6 h. The solvent was removed by a rotary evaporator at 40 °C and 25 Torr. The oil was dried by a stream of nitrogen and stored at -20 °C until used. Fatty acid compositions for hazelnut seed oil were determined using a modified fatty acid methyl ester method as described by Hı ıl (1998). The oil was extracted three times for 2 g air-dried seed sample by homogenization with petroleum ether. The oil samples (50-100 mg) was converted to its fatty acid methyl esters (FAME). The methyl esters of the fatty acids (1 µl) were analysed in a gas chromatography (HP 6890) equipped with a flame ionising detector (FID), a fused silica capillary column (60 m x 0.25 mm i.d.; film thickness 0.20 mikrometere). It was operated under the following conditions: oven temperature program. 175 °C for 7 min. Raised to 250 °C at a rate 5 °C/min and than kept at 250 °C for 15 min); injector and detector temperatures, 250 and 250 °C; respectively, carrier gas. nitrogen at flow rate of 1.51 ml/min; split ratio. 1/50 µl/min. Results of the research were analysed for statistical significance by analysis of variance (Püskülcü and kiz, 1989).

### Results and Discussion

Some physical and chemical properties of hazelnut (Sivri, Tonbul and Kara) varieties collected at different maturation stages were given in Table 1.

Tab. 1. Some physico-chemical properties of three hazelnut cultivars

Harvest periods		Dry matter (%)	Crude ash (%)	Crude oil (%)	Crude protein (%)**
July I	Tombul	59.85±2.17*	1.72	6.51±0.34	10.81±0.78
	Sivri	46.91±1.78	1.65	6.52±0.78	11.48±1.17
	Kara	56.22±2.24	1.65	12.3±1.05	7.60±0.64
July II	Tombul	90.33±3.62	1.38	56.88±2.45	13.33±1.32
	Sivri	90.27±2.89	1.96	54.09±3.41	15.67±1.45
	Kara	94.51±3.56	1.68	52.03±3.09	12.38±1.67
August	Tombul	88.02±1.97	1.74	55.14±1.67	12.46±1.43
	Sivri	81.60±1.67	1.59	58.92±3.78	13.59±1.29
	Kara	78.09±2.71	1.53	51.48±2.34	10.03±0.98
September	Tombul	84.92±2.78	1.69	61.49±2.89	12.15±1.56
	Sivri	94.58±2.54	1.76	53.40±1.78	15.29±1.29
	Kara	94.85±3.42	1.66	66.11±2.86	11.58±1.73

\*mean±standard deviation; \*\*N×6.25

The ash and protein contents of hazelnuts had not been a significant change depending on harvest. The oil contents of hazelnuts were found very low at the first harvest period. The oil contents in this harvest period ranged from 12.3% to 6.51%. The moisture contents of hazelnuts were found low in the same period, too. The protein contents of sharp kind hazelnuts were found higher than the others all the harvest period. The oil contents of hazelnuts were found between 53.40% (sivri) to 66.11 (black) in the last harvest period. Consequently, while protein and ash contents are not change depending on the maturation of hazelnuts, oil content increased. The optimum harvesting time as depending on the physico-chemical properties of the all hazelnut varieties was August and September months. Fatty acid composition of oil of several hazelnut varieties harvested at different periods are presented in Table 2.

Tab. 2. Fatty acid composition of oil of several hazelnut varieties harvested at different periods

Fatty Acidsb (%)	11T MS	11TM T	11TM K	20AS 1	09ET 2	31TM K2	31TM T1	31TM S2	20AT 1	20AK 1	20AT 2	9ES2	9ES1	9EK2	31T MK1	31TM T2	9ET1	20AK 2	31TM S1	20AS2	9EK1
C8:0	-*	-	-	-	-	0.05	0.11	0.09	-	-	-	-	-	-	0.06	-	-	-	-	0.06	-
C10:0	-	-	-	-	-	-	0.05	0.47	-	-	-	-	-	-	-	-	-	-	-	-	-
C12:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	0.03	-
C14:0	0.02	0.16	-	-	0.03	-	0.04	-	0.11	0.03	-	-	-	-	0.03	0.09	-	-	0.08	0.06	0.03
C16:0	4.95	5.29	4.92	4.93	5.76	5.56	7.31	6.35	5.66	5.36	5.42	5.04	5.01	5.03	5.53	5.69	4.93	5.81	5.93	6.17	5.43
C16:1	0.12	0.12	0.11	0.10	0.11	0.11	0.15	-	0.20	0.12	0.12	0.10	0.10	0.09	0.12	0.15	0.11	0.12	0.13	0.12	0.12
C17:1	-	-	-	-	-	-	0.08	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-
C18:0	2.56	2.64	2.36	2.57	2.79	2.86	3.87	2.81	2.31	2.50	2.21	2.40	2.36	2.36	2.84	2.18	2.34	2.86	3.04	2.96	2.18
C18:1CIS	82.79	74.79	85.39	83.68	85.16	85.58	83.95	82.11	76.05	83.89	84.37	85.21	85.23	85.23	85.45	78.76	85.39	85.10	83.91	82.28	84.15
C18:2CIS	8.64	15.64	7.09	8.48	5.90	5.70	4.29	8.17	15.09	7.88	7.76	7.12	7.15	7.19	5.70	12.69	7.10	5.88	6.87	7.99	7.78
C18:3CIS	0.82	1.19	0.12	0.14	0.13	0.14	-	-	0.37	0.13	0.12	0.13	0.15	0.10	0.14	0.29	0.12	0.12	-	0.16	0.21
C20:0	0.10	0.16	-	0.09	0.12	-	0.15	-	0.20	0.09	-	-	-	-	0.12	0.14	-	0.11	-	0.12	0.10
TOPLAM	100.0	99.9	99.9	99.9	100.0	100.00	100.00	100.00	99.99	100.0	100.0	100.0	100.0	100.0	99.99	99.99	99.99	100.00	100.00	100.00	100.00
SAFA	7.63	8.25	7.28	7.59	8.70	8.47	11.53	9.72	8.28	7.98	7.63	7.44	7.37	7.39	8.58	8.10	7.27	8.78	9.09	9.40	7.74
MUFA	82.91	74.91	85.50	83.78	85.27	85.69	84.18	82.11	76.25	84.01	84.49	85.31	85.33	85.32	85.57	78.91	85.50	85.22	84.04	82.45	84.27
PUFA	9.46	16.83	7.21	8.62	6.03	5.84	4.29	8.17	15.46	8.01	7.88	7.25	7.30	7.29	5.84	12.98	7.22	6.00	6.87	8.15	7.99
TRANS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.82	1.19	0.12	0.14	0.13	0.14	0.00	0.00	0.37	0.13	0.12	0.13	0.15	0.10	0.14	0.29	0.12	0.12	0.00	0.16	0.21
6	8.64	15.64	7.09	8.48	5.90	5.70	4.29	8.17	15.09	7.88	7.76	7.12	7.15	7.19	5.70	12.69	7.10	5.88	6.87	7.99	7.78
MUFA+P UFA	92.37	91.74	92.71	92.40	91.30	91.53	88.47	90.28	91.71	92.02	92.37	92.56	92.63	92.61	91.41	91.89	92.72	91.22	90.91	90.60	92.26

\*nonidentified:

TMS: July Sivri, TMT: July Tombul, TMK: July black; AS1: August Sivri 1, ET2: October Tombul 2; TMK2: July Black 2; TMT1: July Tombul 1; TMS2: July Sivri 2; AT1: August Tombul 2; ES2: October Sivri 2; ES1: October Sivri 1; EK2: October Black 2; TMK1: July Black 1; TMT2: July Tombul 2; ET1: October Tombul 1; AK2: August Black 2; TMS1: July Sivri 1; AS2: August Sivri 2; EK1: October Black 1

In general, palmitic, stearic, oleic, linoleic acids were identified as the dominant fatty acids. The oleic acid contents of hazelnuts changed between 74.79% and 85.58% depending on harvest period. Linoleic acid content ranged from 5.70 to 15.64 %, palmitic acid content was ranged from 4.92% to 7.31%. As other fatty acids was found to be minor level. The highest palmitic, oleic and linoleic acid contents were identified respectively in Tonbul (II.harvest), Kara (II.harvest) and Tonbul (I.harvest) varieties. As stearic acid content of hazelnut oils changed between 2.18% (Tonbul, II.harvest) to 3.87% (Tonbul, II.harvest). Content of saturated fatty acids in nuts (SAFA) changed between 7.27% to 9.40%, as monounsaturated fatty acids (MUFA) content changed between 74.91% to 85.69%. There wasn't found trans fatty acid content any harvest period and in variety. The omega-6 fatty acid content of varieties were determined at high rate rather according to omega-3 fatty acid content. The omega-6 fatty acids content of varieties changed between 4.29% to 15.64%. As a result, the progress of ripening has been a minor level reduction oleic acid in varieties. Therefore, fatty acids were shown variability depending on the varieties and harvest time. In the three studied cultivars (Daivana), Total ash contents of hazelnuts were found between 1.87-2.72 % (Köksal *et al.*, 2006). Fat was the major compound, ranging from 56.3% in cv.Daviana to 61.6% in cv. M.Bollwiller. Moisture presented the lower values, between 3.0% in cv. Daviana and 5.6% in cv. F. Coutard (Oliveira *et al.*, 2008).

Oleic acid is the predominant are, ranging from 80.67% in cv. F. Coutard ad 82.63 % in cv. Daviana. Linoleic acid was the second most abundant fatty acid, followed by palmitic and stearic acids (Oliveira *et al.*, 2008). Hazelnut kernels are a good source of fat (50-73%) and contain unsaturated fatty acids (linoleic, linolenic, oleic, palmitic and stearic acids), essential for human health (Garcia *et al.*, 1994). The moisture content of hazelnut cultivars changed between 2.49 % (Black hazelnut) to 5.25% (Cavcava) (Köksal *et al.*, 2006). The fatty acid profiles of all investigated cultivars were consistent with data on Turkish hazelnut varieties (Ba *et al.* 1986; Özdemir *et al.* 2001). Crude protein contents of hazelnut kernels ranged from 11,7% to 20,8% (Köksal *et al.* 2006). Most predominate in the oil of hazelnuts is oleic acid, with amounts between 76.3 % (Do anhisar-Tekke) and 82.6 % (Deutsche), with a mean value of 79.6 % for the five different samples. In nut oils collected in Konya (Beybes-Konya) and Konya (Do anhisar) was more than 76 % of oleic acid found in the oil. As another quantitatively interesting unsaturated fatty acid, the oil contained linoleic acid in a range from 6.5 to 14.0 %, with a mean value of 10.02 %. In the case of linoleic acid, the variation was smaller. In all the other hazelnut oils, the amount of linoleic acid was significantly lower. Nutritionally unfavorable is the high content of saturated fatty acids, consisting of palmitic acid, which amounted to between 5.7 % (Do anhisar-Tekke) and 6.5 % (Beybes-Konya), with a mean value of 6.08 % and stearic acid, which was found in a very small range between 2.1% (Giresun) and 3.8 % (Beybes-Konya), with a mean value of 2.8 % (Matthaus and Özcan, 2012). The main fatty acids of different hazelnut oils in Turkey were 6.38% palmitic, 1.68% stearic, 76.78% oleic and 14.75% linoleic acids (Ba *et al.*, 1986). Allam (2001) reported that hazelnut oil contained 6.91% palmitic, 79.85% oleic, 10.56% linoleic and 2.60% stearic acid. Sixteen fatty acids were identified in Turkish Tombul hazelnut oil, among which oleic acid contributed 82.7% to total, followed by linoleic (8.89%), palmitic (4.85%) and stearic acids (2.73%). Parcerisa *et al.*, (1997) reported that oleic acid was dominant in hazelnut oil. These are comparable to data previously reported in the literature within the limits of the slightly different analytical methods used around the world, the composition of hazelnut oil is uniform. Köksal *et al.*, (2006) established 4.72% to 5.87% palmitic, 0.86% to 2.49% stearic, 74.2% to 82.8% oleic and 9.82% to 18.7% linoleic acids in several hazelnut (*Corylus avellana* L.) varieties. The fatty acid composition of hazelnut oil was similar to that reported previously (Ba *et al.*, 1986; Alasalvar *et al.*, 2003; Özdemir *et al.*, 2001).



## Conclusion

As a result, hazelnut is a good horticulture product, depending on human nutrition and beneficial nutrient composition. Depending on the cultivar and harvest, the oleic acid have been identified the highest rate and have been partial differences among the varieties. The first harvest period is July month that has been harvested two times in this period, one times as in August and September months. In general, the dominant fatty acids have been identified palmitic, stearic, oleic, linoleic acid So, growing conditions, harvest time, storage and processing can affect the nutritional value of hazelnut varieties.

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## **2. PLANT PROTECTION AND FOOD SAFETY**

## RESISTANCE RISK ANALYSIS FOR PLANT PROTECTION PRODUCTS

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### Abstract

Resistance is the naturally occurring, inheritable adjustment in the ability of individuals in a population to survive a plant protection product treatment that would normally give effective control. Although resistance can be demonstrated in the laboratory, this does not mean that pest control in the field is reduced. The importance of resistance depends on the target pest(s) and crop(s), and on the relevance of the product among the available control measures. For this reason, resistance must be determined by using standard test methods and monitored for key pests. Resistance can be seen for insecticides, fungicides and herbicides. Detection of resistance, monitoring and assessment of resistance risk are interrelated. The aim of resistance risk analysis is to describe how the risk of resistance to plant protection products can be assessed and systems for risk management can be proposed in the context of official registration of plant protection products. Resistance risk analysis is a two-stage process, composed of resistance risk assessment and resistance risk management.

**Key Words:** Resistance, risk assessment, risk management, pests, plant protection products

### Introduction

Resistance is the naturally occurring, inheritable adjustment in the ability of individuals in a population to survive a plant protection product treatment that would normally give effective control. Practical resistance is the term used for loss of field control due to a shift in sensitivity (OEPP/EPPO, 1988). Loss of performance of a plant protection product because of the development of practical resistance in the target organism and the subsequent need for additional product use to achieve control can be costly to the grower, the crop protection company and the environment. Registration authorities and crop protection companies now recognize that the development of resistance can be minimized by means of suitable management strategies, and that it is in both their interests to protect the efficacy of plant protection products. The registration procedure, before the product is released for full commercial use, is seen to be the point at which appropriate risk management strategies should be agreed and implemented. For example, the harmonized registration procedure of the countries of the European Union (EU, 1991) requires that applicants provide information on the possible occurrence and development of resistance. If there is evidence to suggest that difficulties of control could result from the development of resistance, a management strategy should be proposed that would minimize the likelihood of resistance.

The aim of resistance risk analysis is to indicate to the registration authorities and to applicants for registration what their obligations are with regard to assessing and managing the risk of practical resistance in the target organism(s). These elements are included in the process of resistance risk analysis:

- the concepts of resistance;
- how resistance risk might be assessed;
- how resistance might be managed;
- what data must be supplied to support the conclusion of a resistance risk analysis;

- other data needed on resistance in the registration dossier;
- reaching a registration decision with regard to resistance risk.

In contrast to detection and monitoring of resistance in the field after the fact, resistance risk assessment is predicting the possibility of resistance emerging as a result of using pesticide in a given use environment.

### **Resistance risk analysis**

Resistance risk analysis is a two-stage process, composed of resistance risk assessment and resistance risk management (OEPP/EPPO, 2002). In resistance risk assessment, the inherent risk is first assessed using the characteristics of the pest and the product; the unmodified risk is then evaluated from the inherent risk when the product is applied under unrestricted conditions of use. In resistance risk management, the decision is made whether the unmodified risk is acceptable; if it is, the process can stop. If the unmodified risk is not acceptable, possible modifiers are then analyzed to determine whether they can be used to mitigate the risk. If suitable modifiers exist, the conclusion of the resistance risk analysis will be a resistance management strategy.

A resistance risk analysis procedure is needed for the different reasons:

- 1) The manufacturer of plant protection products to assess the potential risk of the development of resistance if the product is used commercially.
- 2) The manufacturer of plant protection products to decide which management options should be applied if the assessed risk of resistance is considered to be unacceptable.
- 3) The registration authorities to evaluate any risk assessment submitted by the applicant concerning the development of resistance.
- 4) Registration authorities to evaluate the proposed use pattern suggested by the applicant.

The overall management of resistance is a continuous process, starting with the initial assessment of resistance risk, which must be made during product development, and continuing with the selection of appropriate measures before the start of sales, and with the implementation of the measures throughout the commercial use of the active substance.

### **Resistance risk assessment**

In order to assess the risk of resistance in the target pest(s), it is necessary to evaluate the different factors contributing to the risk, i.e. those inherent in the compound and its effect on the pest and those that might result from a particular use pattern.

The inherent risk depends on various factors, some of which are associated with the product and others with the pest. The factors associated with the plant protection product that may favour the development of resistance can include:

- persistent activity;
- single-site mode of action;
- monogenic resistance;
- ease of metabolism.

Those associated with the characteristics of the target pest that may favour the development of resistance can include:

- short life cycle/many generations;
- high fecundity/widespread distribution of progeny;
- high inherent genetic variability;
- existence of a mechanism in the pest to metabolize a range of active substances;
- existence of cross resistance;
- high fitness of resistant strains.

Past experience may also provide a guide to resistance risk; higher risk could be indicated in situations where a target pest has already developed resistance to other active substances or where resistance to the active substance has already developed in other target pests.

The risk of resistance inherent in the plant protection product and the pest can be increased by certain conditions of use. Agronomic risk affects selection pressure on the development of resistance and is influenced by the particular characteristics of the crop, the geographic area in which the product is applied and the use pattern. The factors influencing the agronomic risk includes:

- widely grown crop with short rotations;
- mono cropping or continuous cropping;
- application techniques;
- other cultural practices (e.g. fertilizers, cultivation);
- need for high numbers of applications or long exposure to obtain control, because of the features of the crop environment;
- use of transgenic plants with genes expressing pesticidal activity;
- use of cultivars susceptible to the pest(s);
- geographic isolation of populations preventing the reentry of sensitive forms;
- environmental conditions favouring more frequent generations or higher population densities of the pest;
- exclusive reliance on a single active substance;
- lack of diversity of available control measures.

Some important factors may influence a resistance risk assessment. These are type of compound, mode of action/mechanism of resistance, cross-resistance, characterization of strains and test methods for sensitivity.

The consequence of resistance will be a reduction in the level of effectiveness of the product, which may ultimately limit the usefulness of the product or of its chemical group. The importance of this will depend on the target pest(s) and crop(s), and on the relevance of the product among the available control measures. In addition, the potential consequences are strongly influenced by the level of resistance in the target pest(s) (i.e. the frequency of resistant strains) and, in particular, by the speed at which the resistance develops.

### **Resistance risk management**

Resistance risk management refers to the process whereby, first, the decision is taken whether the risk of and then, if necessary, conditions of commercial use that have the specific purpose of minimizing or delaying the appearance of resistance in the field are selected and applied. These specific conditions of use are termed ‘modifiers’. If it is accepted that the risk of resistance developing to a plant protection product is proportional to the exposure of the pest to the product, then any modifier which reduces that exposure will reduce the risk of resistance developing. To have any chance of success resistance management should be the collective responsibility of manufacturers, regulatory authorities, advisers and growers. Information on the resistance management strategy can be given to growers/advisers in a number of ways: recommendations and restrictions on use may be included on product labels; advisory literature or use campaigns may also be used.

Having determined the magnitude of the risk of resistance, it is then necessary to decide whether this risk is ‘acceptable’ or ‘unacceptable’. In other words, to decide whether the use pattern should be modified to avoid or slow the appearance of resistance. An acceptable risk is one where the magnitude of the unmodified risk of resistance is considered to be so low, when using the proposed use pattern. Whether a resistance risk is considered to be unacceptable can have important consequences for all sellers and users of a plant protection product, since this decision

determines whether modifiers need to be applied. If the decision about acceptability of risk is wrong, it will lead either to the imposition of unnecessary modifiers or the development of resistance in the target population(s) sooner than could have been hoped.

The acceptability of the risk does not only depend on the magnitude of the risk (the combination of the probability of resistance occurring and the consequences if it does), but should also take account of the benefits to be obtained from the use of the plant protection product. For example, a higher level of resistance risk may be accepted if:

- there is a limited availability of suitable alternative means of control of the target pest(s)
- the plant protection product has advantages over other available products.

There is also specific strategies that can be used in a resistance management strategy.

The integrated use of combinations of different modifiers is likely to be most beneficial. The characteristics of the particular pest/product combination that affect resistance development and have been identified in the assessment of resistance risk should be taken into account when deciding on the exact strategy. In addition, the strategy should take account of the overall pest management in the crop concerned.

Use of good plant protection practice (OEPP/EPPO, 1993) and the specific recommendations for individual crops (OEPP/EPPO, 2001), the amount of plant protection product used can be reduced to what is really necessary.

Measures related to the application of the product, frequency of application, timing, dose rate, mixtures and alternations and recommendations on the product label are also other risk management strategies.

When the risk of development of practical resistance is assessed to be low, but it is nevertheless believed that, in certain rare circumstances, use of the product may still lead to the appearance of an undesirable level of resistance, it may be considered unnecessary to require the implementation of modifiers. In that case, the product label can carry a warning to the user that resistance could occur under certain circumstances and the label could offer general advice, such as that the product should not be used too frequently or should be used in combination with other products.

### Conclusion

The consequence of resistance indicates a reduction in the level of effectiveness of the product, which may limit the usefulness of the product or of its chemical group. Specific details on different types of plant protection products must be considered to determine the resistance. A resistance risk assessment may be made when a new compound is proposed for use on a new target pest or a new crop or environment. Resistance risk assessment is carried out by a variety of personnel associated with pesticide discovery, development or use. After assessed the resistance risk, it is necessary to decide resistance management strategies to reduce the risk of resistance. All information about the resistance risk and risk management must be considered registration of the plant protection product.

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**CONTROL OF *PENICILLIUM EXPANSUM* BY COMBINING *BACILLUS SUBTILIS*  
AND SODIUM BICARBONATE**

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**Abstract**

In the recent years, biological control has been explored as an alternative to the use of synthetic fungicides for managing postharvest decay. Some exogenous substances, such as chitosan, amino acids, carbohydrates, carbonate and bicarbonate salts have been studied to enhance biocontrol capability of antagonists against fungal pathogens. Simultaneous application of chemicals and biocontrol agents could provide more effective means of control and consistent results than that of one approach alone. The objective of the present study was to evaluate and compare the biocontrol efficacy of *Bacillus subtilis* (CFBP 4228) with and without sodium bicarbonate (SBC) against *Penicillium expansum* on apple fruits. The addition of 3% (w/v) SBC in the suspension of *B. subtilis* completely inhibited spore germination of *P. expansum* in potato dextrose broth medium. In combination with *B. subtilis*, SBC exhibited a consistent ability to enhance the biocontrol performance of antagonist against *P. expansum*. Lesion diameter of apple fruits treated with mixture of *B. subtilis* and SBC was significantly reduced, in contrast to inoculation with *B. subtilis* alone. The results of this study show that combination of *B. subtilis* and SCB provided a more effective control on *P. expansum* than applying the antagonist or SBC alone, and can be used as a non-chemical alternative treatment against blue mold on apple fruits.

**Key words:** *Penicillium expansum*, postharvest decay, *Bacillus subtilis*, sodium bicarbonate

**Introduction**

Blue mold caused by *Penicillium expansum* Link is economically important postharvest disease of apple fruits. Conidia of *P. expansum* are present in the orchards, fruit storage rooms, dump-tank and flotation-tank water, and packing facilities. Wounds such as punctures, and bruises on the fruit created at harvest and during postharvest handling are the primary avenue for infection of fruit by this fungus (Rosenberger, 1990). Patulin, a mycotoxin produced by *Penicillium* spp. during fruit spoilage, is a major concern, since exposure can result in severe acute and chronic toxicity, including carcinogenic, mutagenic, and teratogenic effects (McCallum et al., 2002).

Synthetic chemical fungicides, have been traditionally used to control and reducing postharvest diseases (Eckert and Ogawa, 1988). However, the development of fungicide resistance by postharvest pathogens and an increasing environmental concern over fungicide residues in food, have stimulated to find alternative means for controlling postharvest decay (Holmes and Eckert, 1999). Antagonistic biocontrol involves the use of naturally occurring nonpathogenic microorganisms that are able to reduce the activity of plant pathogens and thereby suppress diseases. Antagonistic microorganisms can compete with pathogens for nutrients, inhibit pathogen multiplication by secreting antibiotics or toxins, or reduce pathogen population through hyperparasitism (Mahadatanapuk et al., 2007). Several strains of the genus *Bacillus* produce spores that are resistant to various physical and chemical treatments, such as desiccation, heat, UV

irradiation, and organic solvents, and serve as excellent biological control agents (BCAs) against a wide range of plant pathogens by their production of antibiotics (iturin, surfactin and fengycin), cell wall-degrading enzymes (chitinase and  $\alpha$ -1, 3 glucanase), and antifungal volatiles (Fiddaman and Rossall, 1993; Knox et al., 2000; Leelasuphakul et al., 2008). *Bacillus subtilis* isolated from citrus fruit surface was successfully evaluated for control of citrus green and blue molds caused by *P. digitatum* and *P. italicum*, respectively (Obagwu and Korsten, 2003).

However, BCAs do not have as wide a spectrum of activity under various conditions as fungicides, and most of them cannot achieve the effectiveness of fungicides even under optimal conditions (Conway et al., 2007). Therefore, simultaneous application of several physical and chemical methods could provide more effective means of control and consistent results than that of one approach alone (Zamani et al., 2009). Some exogenous substances, such as chitosan, amino acids, carbohydrates, carbonate and bicarbonate salts have been studied to enhance biocontrol capability of antagonists against fungal pathogens (Depasquale and Montville, 1990; El-Ghaouth et al., 1992; Tian et al., 2002). Among them, sodium bicarbonate (SBC) is effective in controlling postharvest decay of fruits (Smilanick et al., 1999; Karabulut et al., 2001; Yao et al., 2004).

The objective of the present study was to evaluate and compare the biocontrol efficacy of *Bacillus subtilis* (CFBP 4228) and SBC (alone and in combination) against *Penicillium expansum* on apple fruits.

## Material and methods

### Pathogen and Antagonist

*P. expansum* was isolated from decayed apple fruits. The pathogen was maintained on potato dextrose agar (PDA) at 4°C. Conidial suspension of *P. expansum* was prepared as follows: the pathogen was grown on PDA under constant fluorescent light. After 2 weeks incubation at 25°C, spores were harvested by flooding the plates with 10 ml of sterile distilled water containing 0.05% (v/v) Tween 80, scraping with a rubber spatula, and then filtering the suspension through double layers of cheesecloth. The spores were counted with a haemocytometer, and adjusted with sterile distilled water to  $1 \times 10^6$  conidia/ml.

The antagonistic bacterium, *B. subtilis* (CFBP 4228), was obtained from the French Collection of Plant associated bacteria. The bacterial strain was maintained on nutrient agar (NA).

### Effect of *B. subtilis* and SBC on *P. expansum* spore germination

Aliquots of 5 ml potato dextrose broth (PDB) were placed in glass tubes; 100  $\mu$ l of conidial suspension of *P. expansum* ( $1 \times 10^6$  conidia/ml) and 100  $\mu$ l of bacterial suspension of *B. subtilis* ( $1 \times 10^8$  CFU/ml), with or without 1, 2 or 3% (w/v) SBC were added into the glass tubes. All treated tubes were placed in a rotary shaker at 110 rpm at 25°C. After 18 h incubation, 100 spores of fungal pathogen were measured for germination rate and germ tube elongation. Spores were considered germinated when germ tube length was equal to or greater than spore length.

### Biocontrol test

Apple fruits (cv. Golden Delicious) were dipped in ethanol (70%) for 2 min, rinsed with sterile distilled water, air-dried, and punctured with a sterile micropipette tip at the equatorial region (3–4 mm depth). Aliquots of 25  $\mu$ l of bacterial suspension of *B. subtilis* ( $1 \times 10^8$  CFU/ml), and 3% solution of SBC, either alone or in combination, were pipetted into each wound. After 1 h, wound was inoculated with 25  $\mu$ l of conidial suspension of *P. expansum* ( $1 \times 10^6$  conidia/ml). The positive control fruits were inoculated only with fungal conidial suspension, and the negative control with



sterile distillate water. The all apples were placed in moist chamber and incubated at 25°C. After 7 days treatments were compared based on lesion diameters. There were three replicates for each treatment, and the experiment was repeated twice.

#### Data analysis

All data were analyzed by analysis of variance (ANOVA). Mean values were compared using Duncan's multiple range test, and significance was evaluated at  $P < 0.05$ . Statistical analysis was performed using STATISTIKA v.6 (StatSoft, Inc.).

### Results and discussion

Control of *Penicillium* decay of apple fruit is known as very difficult to achieve by biological means, due to the high competitiveness of the pathogen in the wound niche (Wilson and Wisniewski, 1989; Janisiewicz and Korsten, 2002). The effectiveness of antagonist to control diseases caused by *Penicillium* spp. on various fruit improved when they were used with different organic and inorganic additives. Among these additives, bicarbonate salts have showed broad spectrum antimicrobial properties and potential for controlling postharvest decay on various fruits (Smilanick et al., 1999; Karabulut et al., 2001; Yao et al., 2004; Conway et al., 2007). The inhibitory effect of bicarbonate salts on fungal pathogens is probably due to the reduction of cell turgor pressure that results in collapse and shrinkage of hyphae and spores, and consequent inability of fungi to sporulate (Fallik et al., 1997).

The results of this study indicated that conidial germination and germ tube growth of *P. expansum* in PDB was completely inhibited when *B. subtilis* combined with 3% solution of SBC. Zamani et al. (2008) indicated that the presence of SBC in antagonist suspension enhanced the inhibition of spore germination and germ tube elongation of *P. digitatum*. Significant differences were observed among the other treatments and positive control (Table 1).

The result of biocontrol test indicated that 3% SBC was not significant effective in controlling decay incidence of apple fruits caused by *P. expansum* (Table 2). This result was consistent with the observation of Yao et al. (2004), who found that treatment with 2% solution of SBC was not effective in reducing blue mold decay on pear fruits. Biocontrol efficacy of *B. subtilis* was better as compared to SBC, and the disease incidence on antagonist treated fruits was significantly lower than those on the control. However, treatment of fruits with mixture of *B. subtilis* and 3% solution of SBC significantly reduced lesion diameter of apples infected by fungal pathogen. The addition of SBC markedly enhanced the biocontrol activity of *B. subtilis* and showed significantly better disease control in comparison with the antagonistic bacterium alone.

Similar results were observed by Palou et al. (2001), who reported that significant control of *P. italicum* resulted when fruit were treated with 2, 3, and 4% solutions of SBC. Treatment with 1% solution of SBC was ineffective. The SBC treatment was mainly fungistatic, and not very persistent since the fungus survived the treatment. The presence of bicarbonate residues in the wounds was thought to be the cause of the fungistatic effect. The SBC presence delayed spore germination in the treated wounds. Porat et al. (2003) observed that 2% solution of SBC inactivated spore germination of *P. digitatum* in citrus fruit wounds. Also, antagonist *B. subtilis* with SBC improved decay control of *P. digitatum* and *P. italicum* (Obagwu and Korsten, 2003). It was concluded that the space created by the disruption of the pathogen development at the wound site by SBC may have given the antagonist a competitive advantage.

Table 1. Effects of *B. subtilis* and SBC on spore germination and germ tube length of *P. expansum*.

Treatment	Spore germination (%)	Germ tube length (µm)
Control of <i>P. expansum</i>	95.2 ± 2.7* a**	112.3 ± 0.8 a
<i>B. subtilis</i>	46.5 ± 2.1 e	55.5 ± 0.9 e
1% SBC	89.2 ± 1.5 b	90.4 ± 1.3 b
2% SBC	68.9 ± 2.3 c	72.2 ± 1.1 c
3% SBC	58.2 ± 0.8 d	62.3 ± 0.6 d
<i>B. subtilis</i> + 1% SBC	31.3 ± 0.9 f	40.5 ± 1.6 f
<i>B. subtilis</i> + 2% SBC	19.4 ± 1.1 g	27.9 ± 1.4 g
<i>B. subtilis</i> + 3% SBC	0 h	0 h

\*Data represented standard deviations of the means.

\*\*Values in each column followed by a same letter are not statistically different by Duncan's multiple range test (P < 0.05).

Table 2. Biocontrol effects of *B. subtilis* and SBC against *P. expansum* decay on apple fruits.

Treatment	Lesion diameter (mm)
Positive control	34.6 ± 1.5* a**
Negative control	0 e
<i>B. subtilis</i>	18.3 ± 1.1 c
3% SBC	26.3 ± 0.5 b
<i>B. subtilis</i> + 3% SBC	10.0 ± 1.7 d

\*Data represented standard deviations of the means.

\*\*Values in column followed by a same letter are not statistically different by Duncan's multiple range test (P < 0.05).

### Conclusion

Under *in vitro* conditions, SBC had significantly potential to enhance the antifungal effect of *B. subtilis*. Suppressive effects include reduced sporulation and germ tube growth of *P. expansum*. The addition of 3% (w/v) SBC in the suspension of *B. subtilis* completely inhibited spore germination of fungal pathogen in PDB medium. However, the inhibitory effects *in situ* were not as strong as those *in vitro*. The results of biocontrol test showed that combination of antagonistic *B. subtilis* with 3% (w/v) SBC provided a more effective control than the application of antagonist or SBC alone, which should be considered to be a useful and promising measure for controlling postharvest decay of *P. expansum* on apple fruits.

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**CORRELATIVE DEPENDENCIES BETWEEN THE DENSITY AND GROWTH  
PARAMETERS OF *SINAPIS ARVENSIS* (L)**

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**Abstract**

Pot trials were performed in a glasshouse of Institute, during 2011 and 2012. The experiment included five variants with different density of *Sinapis arvensis* L. (1, 3, 6 and 9 plants per plot) with four replicates. The weed height, fresh weight, dry weight and leaf area were determined on 5<sup>th</sup>, 15<sup>th</sup>, 25<sup>th</sup>, 35<sup>th</sup> days after seed germination. The dependence between the number of *Sinapis arvensis* and growth parameters his is summarized by the coefficient of correlation ( $r^2$ ).

It was established that the main parameters determining intraspecific competitive relations in the weed are height, fresh weight, dry weight, leaf area. The growth parameters of *Sinapis arvensis* progressively decreased as the number of weed from 3 to 9 per pot increased. Results of investigation showed that between density of the weed and growth and development his correlative negative dependencies was found. The coefficients of correlation ( $r^2$ ) were very high during the period of studies 5-35 days after germination in all parameters. Marked negative correlations are significant. Height, fresh weight, dry weight, leaf area of weed plants significantly decreased with increasing density of *Sinapis arvensis*. The density of this weeds is one the factors which govern its growth, development and seed production.

Intraspecific competitive relation and correlative negative dependencies between the density of *Sinapis arvensis* and its growth parameters could be useful for examining the biological control of weeds and prediction of yield loss in crop.

**Key words:** *Sinapis arvensis*, density, growth parameters, coefficient of correlation

**Introduction**

Competition between weeds depends on their densities. Intraspecific competitive relations between weeds are a base in the studies of crop/ weed competition.

Increase in the weed number will affect the growth and wield of weed itself through intraspecific effect (de Wit, 2000). *Echinochloa crus-galli*, *Poa annua*, *Setaria viridis*, *Chenopodium album* fresh weight per plant and leaf area were decreased as the density of weed was increased. The dry weight of the weed per plant was reduced as density was higher at higher nutrient levels. More of the dry matter of *Chenopodium album* was allocated to seed when grown at high density (Spiters and van den Bergh, 2002; Williams, 2002). The height, weight of above- ground parts and roots of *Chenopodium album* significantly decreased with increasing density up to six plant per pot. Competition between roots is more important than above-ground competition in *Chenopodium album* (Sattore and Snaydon, 1992). The main parameters determining intraspecific competitive relations in the weeds *Papaver rhoeas*, *Avena fatua* and *Setaria viridis* are: height, fresh weight, dry weight, leaf area per plant. In the period from 10<sup>th</sup> to 40<sup>th</sup> day after seed germination between the numbers of weeds ( 2, 4, 6, 8 at an area of 250cm<sup>2</sup>) and growth parameters a negative correlations was established (Nakova, 2003<sup>a</sup>, Nakova 2003<sup>b</sup>, Nakova, 2004). Competition in annual weeds (*Senecio vulgaris*, *Amaranthus retroflexus*, *Chenopodium album*) significantly increased with increasing the density of weeds up to five per pot. The levels of height, fresh weight, leaf area per plant decreased with competition 4 weeks after germination (O' Donovanl et al., 1995). Campbeli

(1998) found negative correlative dependencies between the numbers of weeds (*Chenopodium album*, *Amaranthus retroflexus*, *Sinapis arvensis*, *Xanthium strumarium*) and their growth parameters. An increase density of weeds 6-14 per pot decreased their levels of the height, above-ground weight, leaf area in early competition 10-20 days after germination. Negative correlation between number of weeds *Viola arvensis*, *Papaver rhoeas* and their growth parameters it was established (Gerovit and Heitefuss, 1996). Weed competition influenced the seed production. It depends on the weed density. The *Amaranthus retroflexus* density of 16 -20 plants m<sup>2</sup> increased the soil seed bank by 14 000- 20 000 seeds m<sup>2</sup>, respectively (van Heemst, 1995; Zimdahl, 1999).

The purpose of the present study was to establish intraspecific competition in *Sinapis arvensis* and correlative dependencies between the density and growth parameters of the weed.

### Materials and methods

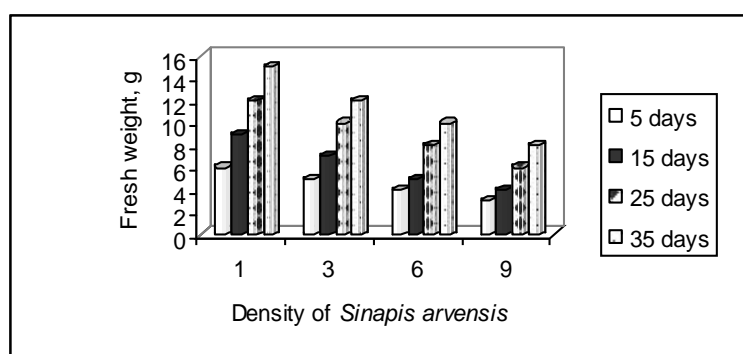
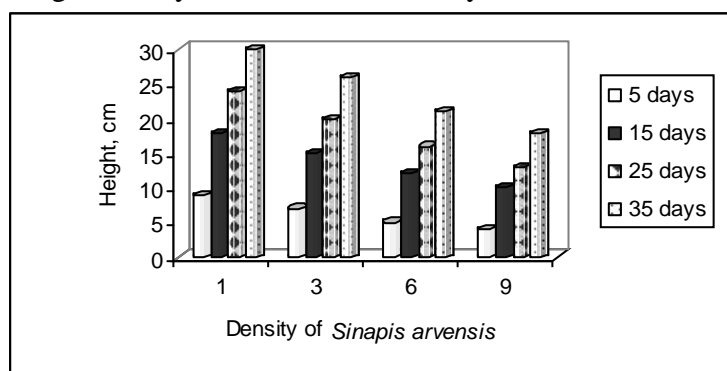
The pot trails were performed in a glasshouse of the Institute in 2011 and 2012. The soil was a sandy clay loam containing 73, 3% sand, 19, 5% clay, 1,07% organic mater and with pH 6,4. The seeds of *Sinapis arvensis* were collected in 2010 and 2011 in the region of Kostinbrod. Their germination was more than 85% in the both years.

Pots of 25 cm diameter and depth of 20 cm, with an area of 500 cm<sup>3</sup> were filled with air dried soil. After germination of *Sinapis arvensis* seedling were thinned to 1, 3, 6, 9 seedlings per plot. The variants were replicated four times. Late emerging *Sinapis arvensis* plants and other weeds were removed by hand. The plants were watered daily. The glasshouse average temperature was 18-22° C. The duration of the trails in 2011 and 2012 was 35 days after seed germination. The *Sinapis arvensis* height(cm), fresh weight(g), leaf area(cm<sup>2</sup>) per plant for each variants were determined on 5<sup>th</sup>, 15<sup>th</sup>, 25<sup>th</sup>, 35<sup>th</sup> days after germination. The growth parameters – leaf area was measured by LICORLI 3100, leaf area meter.

Correlation analyses are employed by statistically processing the data ( SAS, Version 5,0).Correlative dependencies between the density and growth parameters of *Sinapis arvensis* are summarized by the coefficient of correlation (r<sup>2</sup>).The graphs and correlative dependencies are based on the average data over the period 2011- 2012, since data appear uniform over the period.

### Results and discussion

The growth parameters per plant of *Sinapis arvensis* in the period from the 5<sup>th</sup> to 35<sup>th</sup> days after seed germination were significantly reduced as the density of the weed was increase (Figure 1).



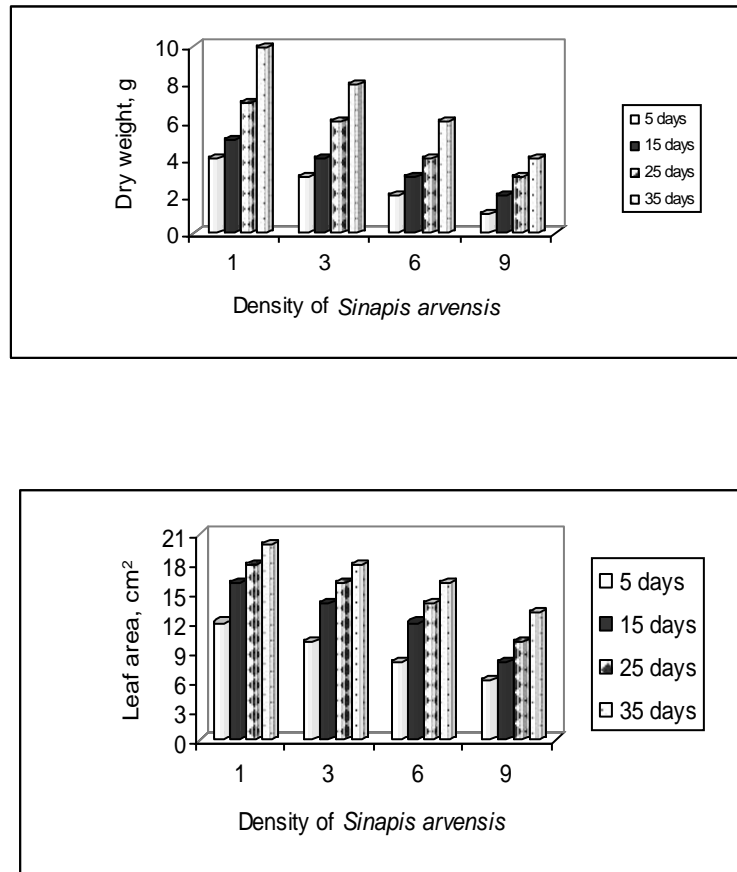


Figure.1 The effect of density on growth parameters of *Sinapis arvensis*

The biggest height, fresh weight, dry weight and leaf area per plant were in pots with one *Sinapis arvensis* plant. The increasing weed density affected negatively on its growth on the 5<sup>th</sup> day after germination. At 3 plants per pots height, fresh weight, dry weight and leaf area of the weed were 78%, 83%, 75%, 83%, respectively, compared with the control (one *Sinapis arvensis* plant per pot). The reduction of these parameters was unidirect in the period 5- 35 days after germination. A greatest effect was found at densities of 6 and 9 plants per pot. Negative effect in the other parameters was established. The presence of 9 plants per pot for the period 5-35 days lowered leaf area by 35- 50% as compared with the control.

Correlative negative dependencies between the density and growth parameters of *Sinapis arvensis* during the studied period were established (Tables 1, 2, 3 and 4). Marked correlation are significant at  $P < 0.05$ . The coefficient of correlation ( $r^2$ ) between number of weeds per pot and height, fresh weight, dry weight, and leaf area per plant varied from -0,94 to -0,98(table 1), from -0,96 to -0,98 (table 2), from -0,97 to -0,99 (table3), from -0,97 to -1 (table 4).

Table 1. Correlative dependencies between density and growth parameters of *Sinapis arvensis* on 5<sup>th</sup> day after germination. Marked correlation are significant at  $P < 0.05$ .

Variables	Weed number	Height	Fresh weight	Dry weight	Leaf area
Weed number	1.0	-0.98*	-0.98*	-0.98*	-0.94*
Height	-0.98*	1.0	0.96*	0.95*	0.98*
Fresh weight	-0.98*	0.96	1.0	0.96*	0.96*
Dry weight	-0.98	0.95*	0.96*	1.0	0.94*
Leaf area	-0.94*	0.98*	0.96*	0.94*	1.0

Table 2. Correlative dependencies between density and growth parameters of *Sinapis arvensis* on 15<sup>th</sup> day after germination. Marked correlation are significant at  $P < 0.05$ .

Variables	Weed number	Height	Fresh weight	Dry weight	Leaf area
Weed number	1.0	-1.0*	-0.98*	-0.98*	-0.96*
Height	-1.0*	1.0	0.98*	0.98*	0.96*
Fresh weight	-0.98*	0.98*	1.0	0.98*	0.96*
Dry weight	-0.98*	0.98*	0.98*	1.0	0.96*
Leaf area	-0.96*	0.96*	0.96*	0.96*	1.0

Table 3. Correlative dependencies between density and growth parameters of *Sinapis arvensis* on 25<sup>th</sup> day after germination. Marked correlation are significant at  $P < 0.05$ .

Variables	Weed number	Height	Fresh weight	Dry weight	Leaf area
Weed number	1.0	-0.99*	-0.99*	-0.98*	-0.97*
Height	-0.99*	1.0	0.97*	0.99*	0.96*
Fresh weight	-0.99*	0.97*	1.0	0.97*	0.98*
Dry weight	-0.98*	0.99*	0.97*	1.0	0.95*
Leaf area	-0.97*	0.96*	0.98*	0.95*	1.0

Table 4. Correlative dependencies between density and growth parameters of *Sinapis arvensis* on 35<sup>th</sup> day after germination. Marked correlation are significant at  $P < 0.05$ .

Variables	Weed number	Height	Fresh weight	Dry weight	Leaf area
Weed number	1.0	-1.0*	-0.98*	-0.97*	-1.0*
Height	-1.0*	1.0	0.96*	0.97*	0.96*
Fresh weight	-0.98*	0.96*	1.0	0.97*	0.98*
Dry weight	-0.97*	0.97*	0.97*	1.0	0.95*
Leaf area	-1.0*	0.96*	0.98*	0.95*	1.0

The result of the experiment showed that the strong competitive effect of *Sinapis arvensis* could be related to weed density. Height, fresh weight, dry weight, leaf area of weed plants significantly decreased with increasing density. A small number of weeds 3 plants per pot had effect on the growth parameters. At density of 6 plants, weed had almost similar effect, but at higher densities 9 plants per pot had higher effect. Similar results on *Xanthium strumarium* were obtained by Kappoor and Ramakrishnan (1998) who showed that various parameters of weed growth

decreased with increase in density. The development and seed production of the weed responded to density stress.

Result indicated that growth parameters of *Sinapis arvensis* progressively decreased as the number of weed plants increased. Height, fresh weight, dry weight, leaf area per plant were reduced as function of weed density.

The work shows that weed density is a major factor in governing weed biomass. Weed biomass production was strongly influenced by density in both years. The weed density governing above-ground biomass and leaf area is important as they will also affect the quantities of weed biomass production.

Between the density of *Sinapis arvensis* and studied parameters negative correlative dependencies in the period 5-35 days after seed germination was established. Increasing the number of weeds per plot decreased the level of height, fresh weight, dry weight, leaf area per plant. The correlation coefficients ( $r^2$ ) for each growth parameters were very high. They confirm the hypothesis of the study of the presence of intraspecific competition between density and growth and development of *Sinapis arvensis*. The correlation coefficients between weed parameters and the number of weeds relatively increased in order: leaf area>dry weight>fresh weight> height. Marked correlations are significant at  $P < 0,05$ . At 95% of weed plants it was established the effect of the density on their reductions of height, fresh weight, dry weight, leaf area in the period of 5-35 days after seed germination.

The work reported that the threshold based on weed density tends to be reliable for general use. This study contributes to a better understanding of the density of weeds as a factor which govern weed growth, development and seed production.

Intraspecific competitive relations and correlative dependencies in *Sinapis arvensis* are a threshold based in the studies of crop/ weed competition.

### Conclusion

Intraspecific competition of *Sinapis arvensis* in the characteristics – height, fresh weight, dry weight, leaf area during the period 5<sup>th</sup>-35<sup>th</sup> days after germination it was established. The growth parameters per plant of *Sinapis arvensis* decreased as weed density (from 3, 6 to 9 per pot with area 500 cm<sup>2</sup>) increased. Negative correlative dependencies between density of *Sinapis arvensis* and growth parameters on 5<sup>th</sup>, 15<sup>th</sup>, 25<sup>th</sup> and 35<sup>th</sup> days after germination was found.

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## PESTS OF APPLE LEAF AND FLOWER BUDS IN THE REGION OF EAST SARAJEVO

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### Abstract

Insect species that feed with leaf and flower buds of apple are significant pests in all areas where this kind of fruit-trees is cultivated. Feeding with buds as imago and/or larvae these insects make damages manifesting in buds drying, disturbing normal development of leaves and flowers, reduction in the yield and quality of fruits.

Exeminations were carried out in apple orchards in three locations on the region of East Sarajevo. Two extensive plantations over 40 years old were situated in the localities of Kasindo and Pale, while one intensive and youngish orchard with sorts Idared, Jonagold and Gold Delicious, was in the locality of Kula. Presence and harmfulness of buds pests was observed in the period of 2007-2008.

Seven species of harmful insects have been reared and determined on apple leaf and flower buds.

Six Lepidopterous species were from the families Tortricidae and Geometridae, and one Coleopterous species was from the family of Curculionidae.

Tortricidae was represented with four species: *Hedya nubiferana* Haworth, *Spilonota (Tmetocera) ocellana* Fab., *Archips podana* Scopoli and *Pandemis heperana* Den.et Schiff.

Two Geometrid species were *Hibernia defoliaria* Clerck and *Cheimatobia brummata* Linne.

Only one beetle from the family Curculionidae was *Anthonomus pomorum* Linne.

In extensive plantations the most numerous species on leaf buds during both years was *Ch. brummata*, but on flower buds it was *A. pomorum*. In intensive plantation on a leaf buds existed 3 species, but two of them (*P. heperana* and *H. defoliaria*) were more numerous, while on a flower buds dominated *A. pomorum* with almost equal representation in all sorts and in both years.

**Keywords:** insect pests, leaf buds, flower buds, apple, East Sarajevo

### Introduction

Apple as the most widespread and economically the most important fruit-tree is attacked by many harmful insects during whole growing season. These insects make damages on different overground organs such as trunk and branches, buds, flowers and fruits. The species which attack leaf and flower buds are very important. Feeding inside or outside of buds they disturb their normal development provoking reduction of growth and fruitfulness, as well quality of fruits. In case of an intensive permanently attack the fruitfulness may be completely absent (Batinica, 1996; Maceljiski, 2002).

Presence and importance of insects and their damages on apple leaf and flower buds is not the same in all areas of their distribution.

The greatest number of such species belong to the family Tortricidae (Lepidoptera), from which the most important pests are *Archips rosana* Linnaeus, *Archips podana* Scopoli, *Spilonata (Tmetocera) ocellana* Fab., *Hedya nubiferana* Haworth, etc. Feeding with buds, leaves and flowers, their larvae

disturb the normal development of vegetative and generative organs, manifesting in reduction of growth and fruitfulness (Batinica, 1996; Maceljiski, 2002).

In Europe, *Archips podana* Scopoli is one of the most harmful leaf roller moths on apples and other fruit-trees, too (Alford, 1999; LaGasa et al., 2003). In Great Britain and North Ireland for example, it is one of three most important harmful leaf roller moth on apple leaves and flowers (Mowat and Clawson, 1996). In early spring larvae feed on buds first, and then on young leaves and fruits (Almaši et al., 2004).

A great damages in nurseries and young apple plantations are provoked by caterpillars of *Hedya nubiferana* Haworth. In Italy and Romania this species is a significant economic pest of buds with damages more than 60% (Rings, 1992; Diaconu et al., 2005). Also, *Spilonota (Tmetocera) ocellana* Fab. can destroy buds on major sprouts, especially in nurseries (Reichart, 1952).

Beside leaf roller moths, leaf and flower buds are usually destroyed by the earliest defoliators in spring, *Hibernia defoliaria* Clerk and *Cheimatobia brummata* Linne (Lepidoptera: Geometridae). In the years of high population densities their larvae can destroy more than 75% leaf and flower buds or provoke completely defoliation which has the negative effects for a fertility in the next year (Maceljiski, 2002).

One of the most important flower buds beetle in our country and enclosed countries is *Anthonomus pomorum* Linne (Coleoptera: Curculionidae). Overwintering adults make damages on closed flower buds during feeding period and oviposition, while larvae feed and development within the buds, provoking their drying and falling (Vukasovic et al., 1965; Maceljiski 2002, Almaši et al., 2004). The level of infestation and damages depend on pest population, length of flowering, fruitfulness and weather conditions during feeding period and oviposition. In some years damages are going up to 70-80% or even more (Maceljiski, 2002; Ciglar, 1998; Cosoveanu, Palagesiu 2010).

The data about pests on apple buds in the region of Sarajevo are relatively old. According to the available literature which data from more than 40 years ago, there were known nine species of leaf roller moths: *Pandemis ribeana* Huebner, *Pandemis heperana* Den. et Schiff., *Archips rosana* Linne, *Archips podana* Scopoli, *Ptycholoma lecheana* Linnaeus, *Croecia holmiana* Linnaeus, *Spilonota ocellana* Den. et Schiff., *Olethreutes variegana* H bner, and *Olethreutes pruniana* H bner (Batinica, 1966).

In East Sarajevo which is a part of Sarajevo's region, the data about harmful insect species on apple is pretty poor. Because of that, the goal of this examination was to establish their presence and number, as well as damages on leaf and flower buds.

### Material and methods

Presence and harmfulness of buds pests was observed in the period of 2007-2008., in field conditions and in laboratory of the Faculty of Agriculture, University of East Sarajevo.

Field experiments have been done in a different apple orchards. Two orchards were extensive, more than 40 years old and situated in localities of Kasindo and Pale. One intensive and youngish plantation with sorts of Idared, Jonagold and Golden delicious, was in the locality of Kula.

Plant material was sampled two times during April and May in bud swelling stage. Each time it was sampled 100 leaf and 100 flower buds in extensive plantations (total number was 200 per year), respectively 100 leaf and 100 flower buds by every sort per year in intensive plantation.

Examination of the presence and number of pests was carried out in laboratory. Sampled insects larvae and pupae were reared to eclosion adults. Rearing was done in a plastic pots with a layer of a cellulose wadding in a bottom which was watered because of bud's freshness. Determination of insect species was done according to morphological features of imago by using an existing keys and adequate entomological literature (Balachowsky, 1966; Hering, 1957).

## Results and discussion

In the region of East Sarajevo, on apple leaf and flower buds, seven insect species were determined. Six species belong to Lepidoptera and one to Coleoptera.

Among six Lepidoptera species, four species were from the family Tortricidae, and two from the Geometridae.

Tortricidae was represented with following species: *Hedya nubiferana* Haworth, *Spilonota (Tmetocera) ocellana* Fab; *Archips podana* Scopoli and *Pandemis heperana* Den. et Chiff.

Geometridae was represented with two species, *Hibernia defoliaria* Clerck and *Cheimatobia brummata* Linne.

From order Coleoptera, only one species, *Anthonomus pomorum* Linne, which belongs to family Curculinidae, was recorded.

In extensive apple orchard in the locality of Kasindo, six species were determined. Three species, *Hibernia defoliaria*, *Ch. brummata* and *Hedya nubiferana* were reared from leaf buds, while four species, *A. pomorum*, *Ch. brummata*, *S. ocellana* and *Archips podana*, from flower buds (Tab.1).

**Tab.1. Insect species and their abundance on apple buds in the locality of Kasindo**

Insect species	2007. year				2008. year			
	23. 04.		08. 05.		28. 04.		14. 05.	
	Leaf buds	Flower buds	Leaf buds	Flower buds	Leaf buds	Flower buds	Leaf buds	Flower buds
1. <i>A.pomorum</i>	0	68	0	17	0	43	0	8
2. <i>H.defoliaria</i>	9	0	5	0	8	0	3	0
3. <i>C.brummata</i>	12	6	8	0	19	9	8	0
4. <i>H.nubiferana</i>	11	0	4	0	6	0	3	0
5. <i>S.ocellana</i>	0	8	0	0	0	4	0	0
6. <i>A.podana</i>	0	5	0	0	0	3	0	0

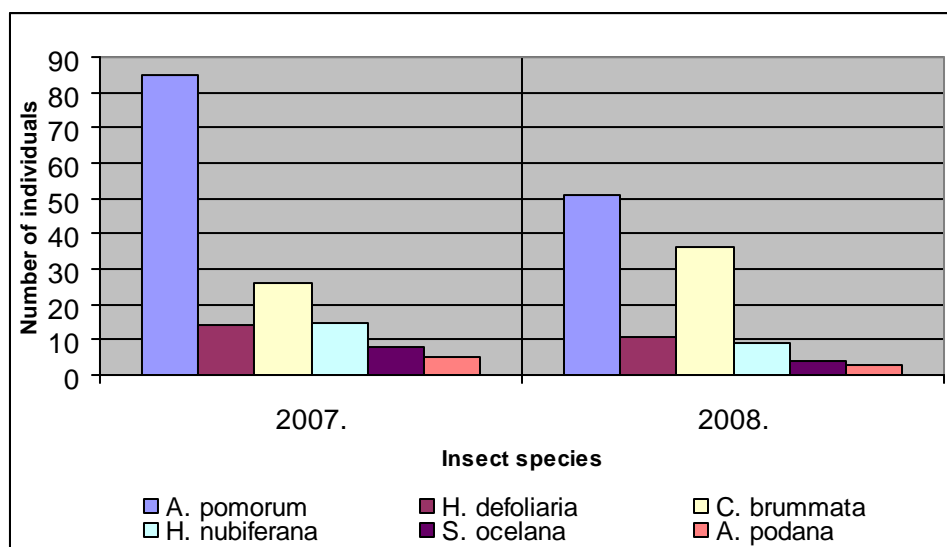
The most numerous species on leaf buds was *Ch. brummata* whose larvae were found in 20 buds in 2007., and 27 buds in 2008. during both sampling. According to 200 surveyed buds per year, damages were from 10% in 2007., to 13,5% in 2008. Greater number of this species in both years were found by first sampling which was done in the third week of April. Untill April 23<sup>th</sup> 2007. it was found in 12 leaf buds (12%), and untill April 28<sup>th</sup> 2008. in 19 buds (19%). In second sampling wich was done untill second half of May, it was less numerous and equal in both years.

The rest two species, *Hibernia defoliaria* and *Hedya nubiferana* were less numerous during both sampling, in both years.

On flower buds, the most numerous species in both years was *A. pomorum*. From 200 surveyed buds in 2007., 85 buds or 42,5% were damaged, respectively 51 buds or 25,5% in 2008. The grater number of this species in both years was in first sampling, at the end of April, before flowering. These results correspond to the literature data about the period of activities and harmfulness of *A. pomorum* which is connected with swelling buds stage (Ciglar, 1998; Maceljiski, 2002; Almaši i sar., 2004; Cosoveanu, Palagesiu, 2010).

The rest three species, *Ch. brummata*, *S. ocellana* and *A. podana*, were found in small number and only during the first sampling.

Total number of all found insects on leaf and flower buds in Kasindo was grater in 2007. related to 2008., except *Ch. brummata*. From all examined species the most numerous in both years were *A. pomorum* on flower buds and *Ch. brummata* on leaf buds predominantly. Following *Hibernia defoliaria* and *Hedya nubiferana* on leaf buds, and *Spilonota ocellana* on flower buds, while *A. podana* was found in the smallest number of flower buds (Graph.1).



Graph.1. The total number of insects on buds in the locality of Kasindo in 2007. and 2008.

In extensive apple orchard in the locality of Pale, six insect species were collected and reared. Three species were reared from leaf buds (*Ch. brummata*, *Hibernia defoliaria*, *Hedya nubiferana*), and four species from flower buds (*A. pomorum*, *Ch. brummata*, *S. ocellana*, *A. podana*). The number of these insects were not numerous as in the locality of Kasindo. More individuals were found in second sampling, actually in first decade of May (Tab. 2). This can be explained by the fact that in Pale an apple orchard is on higher altitude. Because of different climate conditions and lower temperatures, swelling of buds begin later.

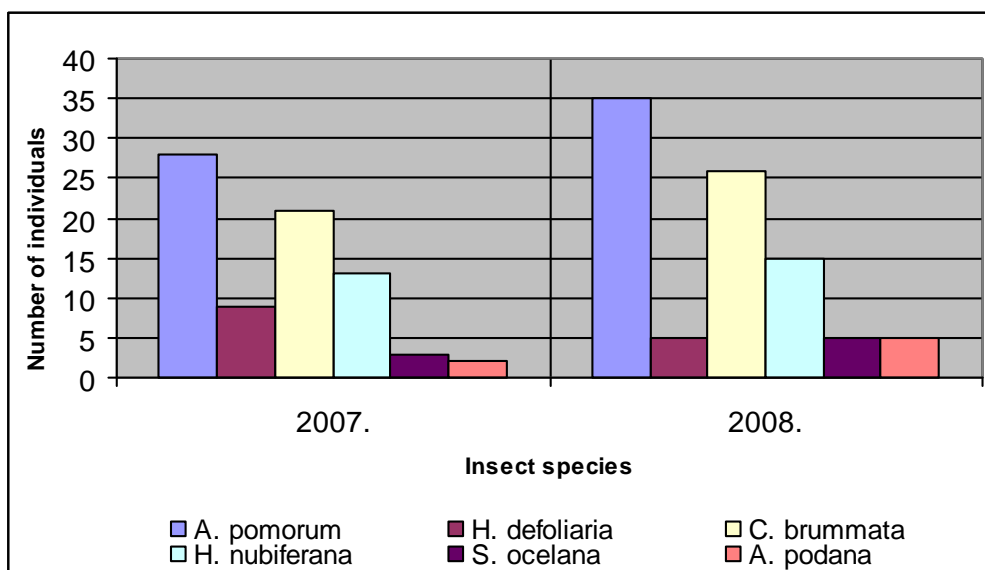
Table 2. Insect species and their abundance on apple buds in the locality of Pale

Insect species	2007. year				2008. year			
	20. 04.		07. 05		23.04		09.05.	
	Leaf buds	Flower buds	Leaf buds	Flower buds	Leaf buds	Flower buds	Leaf buds	Flower buds
1. <i>A. pomorum</i>	0	0	0	28	0	0	0	35
2. <i>H. defoliaria</i>	3	0	6	0	2	0	3	0
3. <i>Ch. brummata</i>	8	1	9	3	7	2	12	5
4. <i>H. nubiferana</i>	4	0	9	0	9	0	6	0
5. <i>S. ocellana</i>	0	3	0	0	0	5	0	0
6. <i>A. podana</i>	0	1	0	1	0	2	0	3

As in previous locality, in this orchard the most numerous species on leaf buds was *Ch. brummata*. According to 200 surveyed buds per year, damages were from 8,5% in 2007., to 9,5% in 2008.

On flower buds, *A. pomorum* exclusively dominated during second sampling in the first decade of May. From 200 surveyed buds in 2007., 28 buds or 14% were damaged, respectively 35 buds or 17,5% in 2008.

Total number of examined insect species on leaf and flower buds in Pale was numerous in 2008. than in 2007, except *H. defoliaria*. The most numerous species were *A. pomorum* and *Ch. brummata*. Following species were *Hedya nubiferana*, *Hibernia defoliaria* and *Spilonota ocellana*. Also in this case *A. podana* was found in smallest number with damages on a single flower buds (Graph. 2).



Graph. 2. The total number of insects on buds in the locality of Pale in 2007. and 2008.

In intensive orchard in the locality of Kula, seven insect species were determined. Four species were reared from leaf buds (*P. heparana*, *Ch. brummata*, *Hibernia defoliaria*, *Hedya nubiferana*) and two species from flower buds (*A. pomorum*, *Archips podana*) (Tab. 3).

Presence of species and their number varied according to different sorts. On Idared, all mentioned species were found on leaf buds, but only one on flower buds (*A. pomorum*). On Jonagold, two species (*Ch. brummata* and *Hedya nubiferana*) were reared from leaf buds, and also one (*A. pomorum*) from flower buds, while on Golden Delicious only one species, *Ch. brummata*, was found on leaf buds, and two species (*A. pomorum* and *Archips podana*) on flower buds.

The total number of specimens, on all sorts, were pretty low in both years. From 100 examined leaf buds of each sort and in every year, number of specimens were from zero to three, while on flower buds it was from two to eleven. The level of damaged flower buds by *A. pomorum* was nearly equal on all sorts, from 9 to 11% in 2007., respectively 6-8% in 2008.

Less insects number on leaf end flower buds in this area can be explained by the fact that this is an intensive plantation with often used agrotechnical, pomotechnical and especial chemical methods because of orchards protection from harmful bioagens.

Table 3. Insect species and their abundance on apple buds in the locality of Kula

Insect species	24. 04. 2007.						23. 04. 2008.					
	Leaf buds			Flower buds			Leaf buds			Flower buds		
	I	J	GD	I	J	GD	I	J	GD	I	J	GD
1. <i>A.pomorum</i>	0	0	0	11	9	10	0	0	0	7	8	6
2. <i>P.heparana</i>	2	0	0	0	0	0	0	0	0	0	0	0
3. <i>Ch.brummata</i>	0	2	0	0	0	0	1	2	3	0	0	0
4. <i>H.nubiferana</i>	1	1	0	0	0	0	0	0	0	0	0	0
5. <i>A.podana</i>	0	0	0	0	0	2	0	0	0	0	0	0
6. <i>H.defoliaria</i>	0	0	0	0	0	0	2	0	0	0	0	0

I- Idared; J-Jonagold; GD-Gold Delicious

## Conclusion

From apple leaf and flower buds seven insect species were collected and reared in the region of East Sarajevo. Determined species were: *Hedya nubiferona*, *Spilonota (Tmetocera) ocellana*, *Archips podana*, *Pandemis heperana*, *Hibernia defoliaria*, *Cheimatobia brummata* and *Anthonomus pomorum*.

In extensive orchard in the locality of Kasindo, the following species were reared from leaf buds: *Hibernia defoliaria*, *Ch. brummata* and *Hedya nubiferana*. The most numerous was *Ch. brummata*, with damages of leaf buds from 10% to 13,5%.

Among four species on flower buds (*A. pomorum*, *Ch. brummata*, *S. ocellana*, *Archips podana*), *A. pomorum* was most significant with damages from 25,5% to 42,5% .

In extensive orchard in the locality of Pale, from three species on leaf buds (*Ch. brummata*, *Hibernia defoliaria*, *Hedya nubiferana*), the most numerous was *Ch. brummata*, whose larvae were damaged 8,5% to 9,5% of leaf buds.

On flower buds, among four detected species (*A. pomorum*, *Ch. brummata*, *S. ocellana*, *A. podana*), the most numerous was *A. pomorum*, with damages 14%-17,5%.

In intensive plantation in the locality of Kula, there were four species on leaf buds (*P. heparana*, *Ch. brummata*, *Hibernia defoliaria*, *Hedya nubiferana*), and two species on flower buds (*A. pomorum*, *Archips podana*). All of them were found on a single buds of all sorts, except *A. pomorum*, which was the most numerous with nearly equal damages (6% -11%) on all sorts and in both years.

The abundance of registered insect species and their damages on leaf and flower buds were grater in extensive apple orchards than in intensive one.

In all plantations and localities, the most significant pests were *Cheimatobia brummata* on leaf buds, and *Anthonomus pomorum* on flower buds.

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## RESIDUES FOLPET IN GRAPE ŽILAVKA AND BLATINA VARIETIES FROM PLANTATION CULTIVATION AND IN THE SMALL VINEYARD

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### Summary

The application of pesticides is a necessary measure in the cultivation of grapevine. Fungicides, in comparison to other pesticides, are the most often used in vineyards due to the sensitivity of grapevine on the most common diseases caused by pathogenic fungi (*Plasmopara viticola*, *Uncinula necator* and *Botrytis cinerea*), which reduce the yield and quality of grapes. Frequent use and misuse regardless respecting of preharvest period, causes accumulation of fungicide active substances in grapes, and then their transfer into the wine. In this paper the results of the residues of folpet in two most frequently grown varieties of wine grape (Žilavka and Blatina) from plantation cultivation and in the small vineyard are presented. The device GC/MS Agilent 7890A/5975C and analysis method UNI EN 15662:2009 QuEChERS was used for determination of residues of folpet. Determined concentrations of residues of folpet in grapes of Žilavka and Blatina grown in 2011 in two plantations and in the small family vineyard were below the MRL value (Maximum Residue Levels) determined for folpet with Regulation (EC) No. 396/2005, which for wine grapes is 5 mg / kg as well with Regulations on the quantities of pesticides and the other toxic substances, hormones, antibiotics and mycotoxins that may be present in food ("Official gazette of SFRJ", No. 59/83 and 79/87) that for folpet is 2 mg/kg.

**Key words:** grapes, folpet, MRL, Žilavka, Blatina

### Introduction

Use of fungicides has special significance in successful control of the most often grapevine diseases (*Plasmopara viticola*, *Uncinula necator* and *Botrytis cinerea*). The most commonly used fungicides for grapevine protection are procymidone, cyprodinil, fludioxonil, myclobutanil, iprodione, folpet, vinclozolin (Flamini and Ponighel, 2006). Excessive use of fungicides indicates necessity for control of their residues in grapevine and wine. In Europe, grapevine is food that is the most contaminated with pesticide residues. The report published by Environmental Working Group (EWG) for 2010 indicate that grapevine is in the group of 12 food items with the most often reported pesticide residues. Residues of these compounds are one of the most important contamination sources in production of fruits and vegetables, and may present hazard for health of consumers and environment. The studies published in 2007 shows that grapevine is among 24 different food items analysed within annual food monitoring EU programmes in the period 2001-2005 and it holds fourth place considering fungicides residues quantity after pears, oranges and strawberries<sup>2</sup>. According to literature related to problems of fungicides residues in grapevine during harvest and during wine production, folpet residues are the ones that the most commonly may be found. Results of the study Baša - esnik et al. (2008) regarding monitoring of pesticide residues in 47 grapevine samples showed the most often presence of folpet (97,9%), then cyprodinil (51,1%), dithiocarbamates (44,7%), hlorotalonil (23,4%), chlorpyrifos (19,1%) and pyrimethanil (14,9%).

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<sup>2</sup> Internet, Annual EU-wide Pesticide Residues Monitoring Report – 2001-2005, European Commission (2007)

Soleas and Gilbberg (2000) analysed 26 pesticides in 1827 samples of must prepared for fermentation, and in 1537 vine samples, from nine world regions (Australia, Canada, Central Europe, France, Italy, South Afrika, Spain and USA), folpet (14,5%) and captan (13,9%) were mostly detected, followed by carbaryl (7,9%), malathion (3,5%) and dimethoate (2,5%). Folpet residues in squeezed white grape of „Godello“ variety, were 0,34 mg/kg and they were below the limit of vine determination that is under EU MRL (5 mg/kg) (Gonzalez-Rodriguez, 2011). During vinification process folpet is quickly degraded by photo-lytic and hydro-lytic reactions (Cabras et al., 1997 – according to uš et al., 2010b). Results of study presented by Mlikota (1993) showed that alcoholic fermentation of grapevine must is influenced by folpet residues, while vinclozolin, procymidone and dihalofluanid do not effect it at all. All fungicides had good reaction on grey mold and residues were below. Mlikota et al. (1996) implies that fungicides of older generation, such as folpet and captan, have negative influence on fermentative activity of wine yeast. According results presented by uš et al. (2007), who monitored concentration and dynamics of active substance degradation in grapevine produced by integrated production principles, all folpet concentrations in white grapevine variety were below MRL. For red grapevine variety, folpet concentration in the amount of 0,08 mg/kg was detected in the phase of grape pulp, while in all later samples concentration was not over the determination limit. Same authors (2010) were investigating during 2007, the content of pesticide residues in Slovenia in two white (Malvasia and Pinot Gris) and two red varieties (Blaufränkisch and Refosco) of grape during grape ripening and through vinification process. Residue concentrations were decreasing in three varieties (Malvasia - 1,99 > 0,58 > 0,10 mg/kg; Pinot Gris - 2,77 > 1,23 > 0,68 mg/kg and Refosco – 3,39 > 1,27 > 0,41 mg/kg). Folpet was determined in must of white variety Pinot Gris, through vinification process in the concentration of 0,04 mg/kg, while it was not determined later on. Residues of folpet in Refosko variety were determined in squeezed grape and they were in the amount of 0,20 mg/kg. Results of Rial Otero et al. (2004) who analysed samples of grape on residues of 15 fungicides, showed presence of folpet in 2 samples in concentrations of 0,1 and 0,4 mg/kg which is below national MRL (Spain). Vinification process decreases pesticide level and their content in vines is significantly lower in vines comparing to fresh grapes. Consequently, methods for detection of residues pesticide have to be very efficient and sensitive (Wong, Halverson, 1999). Unterweger et al. (1997) described GC/MS method for folpet residues detection in grape juice, must and vine.

The aim of this paper was detection of presence and concentration of a residue from active substance folpet in grape varieties Žilavka and Blatina which are grown in plantation vineyard and in the small family vineyards in the wider area of the city Mostar.

### Material and methods

The research was conducted in 2011, in vineyards within the area of the city of Mostar, at four locations. Three of them (Hum ine, Mukoša and Žitomisljic) are plantations, while location Hodbina is small family vineyard (Table 1). In this study we are investigated the presence of folpet residues in Žilavka and Blatina, the most common grape varieties in Herzegovina.

Table 1. Basic data on vineyards whose grape was sampled

Location	Vineyard surface	Vineyard age	Grape variety
1. Hum ine	2 ha	9 years	Žilavka and Blatina
2a. Mukoša	13 ha	5 years	Žilavka
2b. Žitomisljic	30 ha	7 years	Blatina
3. Hodbina	0,4 ha	6 years	Žilavka and Blatina

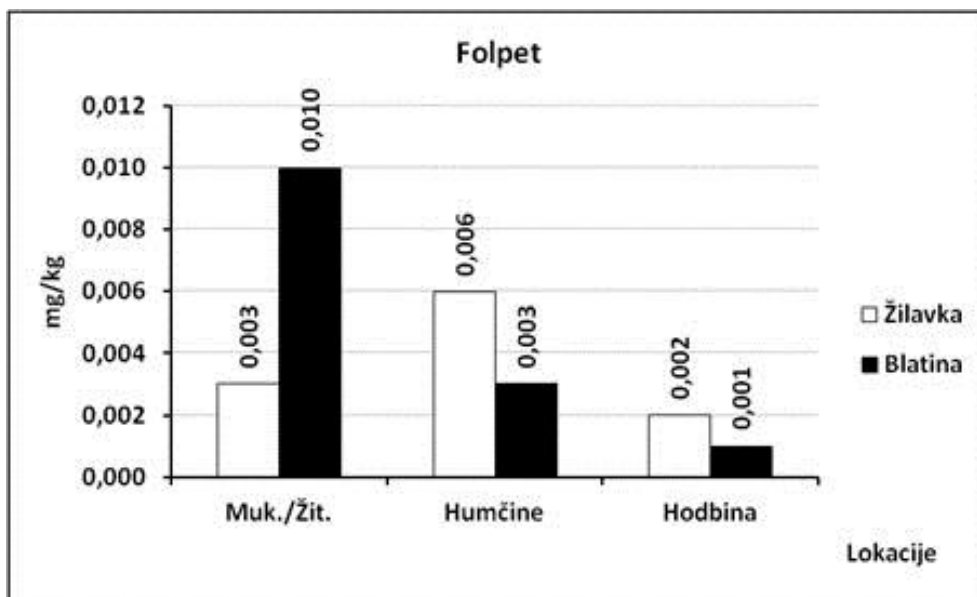
Sampling grape was done during the ripening period. The average sample was taken from five grapevines with different heights and foreign vines. The average weight of every sample was around 2 kg, with 5 bunches at least. The samples were placed into plastic bags and stored in portable refrigerator before delivering to the laboratory. The content of sugar in grape was determined on the spot, using hand refractometer 32 ATC. Grape samples which were chosen for determination of total acidity, were separate packed in plastic bags and transported to the laboratories of Federal Institute for agriculture in Sarajevo for further analyses. Determination of residues was also carried out in the same laboratory. Analytic standard of folpet was purchased at Mikro+Polo company. Characteristics of used standard were: Folpet – Sigma Aldrich, 250 mg; Batch: SZBA 0,25×V; Cleanliness: 99,7%. In term to determine concentration of folpet residue, GC/MS Agilent 7890A/5975C device was used as well UNI EN 15662:2009 QuEChERS analyses method. Basic characteristics of method which were used for this research are: Column: GC DB SMS; Port Number 190915-433; Length (m) 30 Diam (mm) 0,250 Film (µm) 0,25; Injector: Split-Splitless Inlet 280<sup>0</sup>C; Colon pressure: 21,719 psi; Total Flow: 19,992 ml/min; Chromatography period (Run Time): 38,517min. The protection program grape based on folpet at the examined locations, is presented in the Table 2.

Table 2. Folpet program protection for grape varieties Žilavka and Blatina at surveyed localities

LOCATION									
No.:	Hodbina			Humčine			Mukoša/Žitomisljic		
	Preparation	Active substance	Application (kg/ha)	Preparation	Active substance	Application (kg/ha)	Preparation	Active substance	Application (kg/ha)
1.	FANTIC F	Benalaksil 4% + folpet 48%	2,5	FANTIC F (2×)	Benalaksil 4% + folpet 48%	4,9	PERGADO F	Mandipropamid 5% +40% folpet	2
2.				FOLPAN	Folpet 50%	2,5	FORUM STAR	Dimetomorf 11,3% + folpet 60%	2
3.							MIKAL PREMIUM F	Fosetil 50% + 25% folpet + 4,1% iprovalikarb	3

### Results and discussion

Results of our survey regarding contents of folpet residues in grape varieties Žilavka and Blatina are presented in Graph. 1.



Graph 1. Contents of folpet residues (mg/kg) in grape varieties Žilavka and Blatina in locations Mukoša/Žitomisljic, Hum ine and Hodbina in 2011

Contents of folpet residues in grape samples (Žilavka and Blatina) from locations Hum ine and Hodbina were ranged from 0,001 to 0,006 mg/kg. Samples of Blatina variety from location Žitomisljic contained the highest residues of folpet (0,010 mg/kg). Samples of Žilavka variety from locations Hum ine and Hodbina contained double higher concentration of folpet residues in comparison with Blatina variety. Taking into account locations/vineyards, the highest concentration of folpet residues was detected in location Mukoša/Žitomisljic (0,0065 mg/kg), while the lowest were detected at the location in Hodbina (0,0015 mg/kg). Grape variety Blatina contained averagely it contained more folpet residues (0,0047 mg/kg), comparing to the grapes of Žilavka variety (0,0037 mg/kg).

The protection program grape in the vineyard plantation Žitomisljic was the same as the program was conducted in the vineyard plantation Mukoša. Regarding the same group of agronomists together with same applied agro-technical measures, in order to fulfil all demands necessary for correct analyses of variance, vineyards from Mukoša and Žitomisljic were treated as one vineyard/location. Results of analyses of variance of folpet residues are present in Table 3.

Table 3. Analyses of variance of content of folpet residues (mg/kg) in grape varieties Žilavka and Blatina in 2011 in locations Mukoša/Žitomisljic, Hum ine and Hodbina

Analyses of variance					
Variability source	Deviance	Degrees of freedom	Variance	F <sub>exp</sub>	F <sub>tabl.0,05</sub>
Cultivar	0,00001	1	0,00001	0,49	4,41
Location	0,00009	2	0,00005	2,37	3,55
Interaction	0,00012	2	0,00006	3,26	3,55
Error	0,00034	18	0,00002		
Total	0,00057	23			

Results of analyses of variance showed that location of vineyard (Mukoša/Žitomislic, Hum ine and Hodbina ) and grape variety (Žilavka and Blatina) didn't have statistically significant influence on concentration of folpet residues.

### Conclusion

According results of this survey, it can be concluded that determined concentrations of folpet residues in grape varieties Žilavka and Blatina were far below MRL (Maximum Residue Levels) determined for folpet through the Regulation (EC) No 396/2005<sup>3</sup> and Regulations on quantities of pesticides and the other hazardous substances, hormones, antibiotics, and mycotoksins that may be present in food („Official Gazzette SFRJ“, No.: 59/83 and 79/87). This regulation as MRL for folpet in grapevine determines 5 mg/kg, while 2 mg/kg is allowed in the accordance to the SFRJ regulations. Additionally, determined concentrations of folpet residues are significantly below the values that are mentioned in numerous publication that dealt with this issue.

Study indicated appropriate application of folpet, without consequences to the residues in grapes and its processed products. However, constant work with grape producers, adoption and implementation of quality regulations and with development of analytic- research infrastructure, is necessary to insure constant supervision over fungicide residues and the other means used for protection of grapes.

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## EFFECT OF SOWING DATE ON SPECIES COMPOSITION OF INSECT PESTS ON WINTER TRITICALE DURING THE SPRING AND SUMMER IN BULGARIA

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### Abstract

The effect of sowing date of winter triticale on the species composition of insect pests on the crop during the spring and summer growth stages was evaluated in Kostinbrod (Bulgaria) for the period 2009 - 2013. Forty-two damaging insect species belonging to six orders: Orthoptera, Thysanoptera, Hemiptera, Coleoptera, Hymenoptera and Diptera, were identified. In early spring (during the growth stages of tillering and stem elongation) the most damaging species for early winter sowings (sown during the last decades of September) were stem boring pests. Their complex included adults of Hemiptera, and larvae of Coleoptera and Diptera, among which *Opomyza florum* and *Phorbia fumigata* were the most important species. Damages caused by *O. florum* were prevalent in early sowings, while those caused by *P. fumigata* were more pronounced in late triticale sowings (sown in the second and third decade of October).

In late spring and early summer (during the growth stages of heading – ripening of winter triticale) the most damaging insects were sap-sucking species from the orders Thysanoptera and Hemiptera, and family Cecidomyiidae (Diptera). The wheat thrips *Haplothrips tritici* was the most abundant. Twenty five species of Hemiptera from the families Aphididae (3 species), Aphrophoridae (1 species), Cicadellidae (9 species), Delphacidae (1 species), Miridae (5 species), Pentatomidae (4 species) and Scutelleridae (2 species) were found to damage the leaves, spike and grains of winter triticale. The dominant species were *Aelia rostrata* and *Eurygaster maura*. Insect pests from the genera *Dolerus* and *Cephus* were of insignificant importance.

**Key words:** growth stages of winter triticale, insect pests, sowing date, damages

### Introduction

Triticale is a new promising cereal crop with high potential fertility and valuable nutritional quality of the grain. For the past three years in Bulgaria, its area for cultivation has increased from 8590 ha to 12 000 ha and triticale is currently ranked fourth with respect to total growing area (after wheat, barley and oats). With respect to yield, triticale is ranked third, after wheat and barley (Agrarian reports, 2010, 2011, 2012). Fulfilling the potential of the crop is highly dependent on the application of modern technology for its cultivation, providing optimal conditions for the growth and development of plants. An important agricultural factor is the period of sowing, which could substantially change the plant health status of the crop through its influence on the distribution, density and activity of damaging phytophagous insects.

In the entomological literature, the data on the species composition of the insect fauna of triticale are scarce. The triticale pest list of the Republic of Belarus includes representatives from 6 orders: Hemiptera, Coleoptera, Thysanoptera, Diptera, Hymenoptera and Lepidoptera. In sowings of winter and spring triticale, the authors have established identical species composition of the pests, but different structure and abundance of the dominant species. In winter crops, the dominant species are *Sitobion avenae*, *Haplothrips aculeatus*, *Limothrips denticornis* and *Oscinella* sp. while in spring crops these are *Oscinella* sp, *Oulema melanopus* and *Rhopalosiphum padi* (Prohorova et al., 2000). According to Larsson (2005) two thrips species are common on triticale in southern Sweden:

*Limothrips denticornis* and *Thrips angusticeps* but *T. angusticeps* is not a significant pest. *L. denticornis* was found to cause the highest yield loss on triticale among the thrips species.

The data on the pest insect fauna of triticale in Bulgaria are scarce. The species and quantitative composition of the most important stem pests in the autumn and early spring growing seasons was studied (Krusteva and Ventsislavov, 2006; Krusteva et al., 2006; Krusteva and Karadjova, 2011). The pest complex includes larvae of Elateridae, *Chaetocnema* and Diptera, larvae and adults of Heteroptera. The insect pests of greatest importance in the autumn growing season are *Phorbia fumigata*, *Oscinella frit* and *O. pusilla*, and during the early spring period, these are *Opomyza florum* and *Phorbia haberlandti*. The aim of the present study is to obtain data on the species composition of the insect fauna during the late phenological stages of triticale in spring and early summer in relation to the sowing date. This information is necessary in order to develop monitoring and pest management programmes for winter triticale in Bulgaria.

### Materials and methods

The study was conducted in the experimental fields of the Institute of Soil Science, Agrotechnology and Plant Protection in Kostinbrod, Bulgaria with winter triticale variety *Vihren*. The crop was sown on September 25<sup>th</sup>, October 9<sup>th</sup> and October 26<sup>th</sup> (2009), September 26<sup>th</sup> and October 21<sup>st</sup> (2011), and September 28<sup>th</sup> and October 16<sup>th</sup> (2012). The population dynamics of leafhoppers (Cicadellidae, Delphacidae, Aphrophoridae), plant bugs (Miridae, Pentatomidae, Scutelleridae), adults of Chrysomelidae leaf beetles (*Lema cyanella*, *Oulema melanopus*, *Phyllotreta vittula*), cereal flies (Opomyzidae, Chloropidae and Anthomyiidae), Orthoptera and Hymenoptera were monitored using a sweep-net (5 sets of 20 sweeps, d=300 mm) in each surveyed field from early spring seedling emergence (from March - beginning of April, depending on the weather conditions) until harvest. Samples were collected once a week. The rule of “5 sweeps = 1m<sup>2</sup>” was used to calculate the number of flies/m<sup>2</sup> (Mihailova et al., 1982). After the first symptoms of damage were observed (withering and yellowing of the main leaf) triticale plant samples of 0.5 linear meters were regularly collected and analyzed in the laboratory in order to establish the species composition of the pests, damaging triticale shoots. Damaged shoots were dissected and the phenophase and type of damage were documented. The pest species and their life stages were described. The percentage of damaged shoots per sample was calculated. Analyses of plant samples were carried out in 4-6 replicates in the phenophase of tillering and stem elongation (DC 21-39 on Zadoks growth scale) (Zadoks et al. 1974).

The population dynamics of Aphididae and larvae of *L. cyanella* and *O. melanopus* were monitored by counting insects on 100 stems (collected at 10 different locations, 10 stems per location) at 3-5 day intervals from inflorescence emergence until milk development (DC 50-77). The population dynamics of adults and larvae of Thysanoptera and larvae of *Sitodiplosis mosellana* were monitored by counting insects on 20 ears at 5-7 day interval during growth stages DC 50-85. The number of Aphididae and larvae of *L. cyanella* and *O. melanopus* per stem, as well as the number of adults and larvae of Thysanoptera and larvae of *S. mosellana* per ear were calculated. The percent of damaged grains by larvae of Thysanoptera and *S. mosellana*, and adults and larvae of Pentatomidae and Scutelleridae was established by counting the damaged grains on 30 ears during phenophases DC 85-92.

The species identification was performed as follows: for Aphididae after Blackman, Eastop (1989) and Emden (1972), for Thysanoptera after Zur Strassen (2003) and Schliephake and Klimt (1979), for Diptera after Beshovski (1985), (Kopaneva, 1980) and Hennig (1976), for Hymenoptera and Orthoptera after Kopaneva (1980), for Heteroptera by comparison to a collection identified by Yosifov, Bulgarian Academy of Science (BAS) and for Hemiptera (Aphrophoridae and Cicadellidae) using a collection, identified by Viola Bayryamova (BAS) and Venelin Pelov



(Institute of Soil Science, Agrotechnology and Plant Protection). The species composition of wireworms (Elateridae; Coleoptera) was not identified, only the percentage of shoots damaged by their larvae was calculated.

### Results and discussion

During the period of the study, 42 species of insect pests from six orders were identified. Their relative abundance, density and degree of damage are closely connected to the phenological development of the triticale sowings and the climatic conditions. During early spring (DC 21-39), the sowings were infested by 24 species of insects, which include stem-boring, leaf-chewing and sap-sucking pests (Table 1). The most damaging group which comprises the highest number of species (13) is that of stem pests: adults of Miridae and Pentatomidae, larvae of Elateridae, *Chaetocnema aridula*, *Ch. hortensis*, and dipterans from the families of Opomyzidae, Chloropidae and Anthomyiidae

Table 1. Species composition of harmful insects on winter triticale during the growth stages of tillering and stem elongation of the crop (DC 21-39 on Zadoks growth scale)

Group of pests	Order	Family	Species	
Stem pests	Hemiptera	Miridae	<i>Lygus rugulipennis</i> Poppius 1911 <i>Stenodema virens</i> (Linnaeus 1767)	
		Pentatomidae	<i>Aelia acuminata</i> (Linnaeus 1758) <i>A. rostrata</i> Boheman 1852	
	Coleoptera	Elateridae	<i>Agriotes</i> spp.	
		Chrysomelidae	<i>Chaetocnema aridula</i> (Gyllenhal 1827) <i>Ch. hortensis</i> (Geoffroy 1785)	
	Diptera	Opomyzidae	<i>Opomyza florum</i> (Fabricius 1794)	
		Chloropidae	<i>Elachiptera cornuta</i> (Fallen 1820) <i>Lasiosina brevisurstylata</i> Dely-Draskovits 1977 <i>Lasiosina herpini</i> (Guerin-Meneville 1843) <i>Oscinella frit</i> (Linnaeus 1758)	
		Anthomyiidae	<i>Phrobia fumigata</i> (Meigen 1826)	
		Coleoptera	Chrysomelidae	<i>Lema cyanella</i> (Linnaeus 1758) <i>Oulema melanopus</i> (Linnaeus 1758) <i>Phyllotreta vittula</i> (Redtenbacher 1849)
		Hemiptera	Aphididae	<i>Schizaphis graminum</i> (Rondani 1852) <i>Sitobion avenae</i> (Fabricius 1775)
	Cicadellidae		<i>Balclutha punctata</i> (Fabricius 1775) <i>B. rhenana</i> Wagner 1939 <i>Empoasca pteridis</i> (Dahlbom 1850) <i>Hardya anatolica</i> Zachvatkin 1946 <i>Psammotettix provincialis</i> (Ribaut 1925)	
	Delphacidae		<i>Laodelphax striatellus</i> (Fallen 1826)	

The results of the performed analyses show that among the stem pests, dipterans have greatest economic importance for triticale in early spring. During the period of the study, 6 species of order Diptera were identified. The percentage of stems damaged by dipteran larvae varied from 32% in early sowings to 42% in late sowings. The prevalent damages in the early sowings were caused by *O. florum* (31-38%), while damages from *Ph. fumigata* were predominant in the late sowings (11-

22%). The rest of the species in the group of stem pests have limited importance for winter triticale and did not show any significant differences in damage in relation to the sowing date: Elateridae sp. (0-1.6%), *Ch. aridula* and *Ch. hortensis* (0-2.4%) and Heteroptera (0-2.30%). The species belonging to the groups of leaf-chewing (*L. cyanella*, *O. melanopus*, *Ph. vittula*) and sap-sucking insects (Aphididae, Cicadellidae and Delphacidae) have no economic importance for the crop during early spring and were established at densities below 1 individual / m<sup>2</sup>.

The insect pest fauna during the later phenophases of the development of triticale, from the end of booting to ripening (DC 40-92), established during the years of the investigation includes 34 species from 6 orders (Table 2). The group of sap-sucking insects is represented by the highest number of species and shows greatest overall abundance. It includes insects from the orders Thysanoptera, Hemiptera and Diptera. Two species from order Thysanoptera were established, of which *H. tritici* was dominant. It was the most abundant and persistent pest in triticale sowings. In 2013 mass development of *H. tritici* adults was established at the beginning of heading (DC 45-55), while the abundance of larvae was highest during the stages of early milk to soft dough (DC 73-85). There were no significant differences in the peak densities of *H. tritici* adults and larvae in relation to the sowing date (11 versus 10 adult individuals per ear; 36 versus 40 larvae per ear). In 2010, the larvae of *H. tritici* were developing together with the larvae of *S. mosellana*. They suck sap from the stamen, ovary and grains during milk and dough development. One to 4 (rarely 8) larvae of *H. tritici* and 1-13 larvae of *S. mosellana* were found to develop in one grain. Grains with 1-2 larvae of *S. mosellana* were most common. The results of the performed analyses showed that the relative quantity of damaged grains per ear varied from 15.3 to 41.6% for *H. tritici* and from 0.25 to 4.9% for *S. mosellana* in the early and late sowings, respectively. At these densities of the larvae, the quantity of severely damaged grains was 8.6-14.3%. The interior of such grains is completely destroyed and they are discarded during harvest.

The data on the species diversity and density of Hemiptera do not show significant differences in relation to the sowing date. Ten species from two families were established: Aphrophoridae (1 species) and Cicadellidae (9 species). Three species from genus *Psammotettix* were prevalent: *P. provincialis*, *P. striatus* and *P. alienus*. The peaks in the overall abundance of leafhopper pests in the crops with different sowing dates during the period of the investigation did not exceed 5 individuals/m<sup>2</sup> at the phenophase of dough development (DC 83-85) in the first decade of July. *Philaenus spumarius* was second in importance in the fields during 2012. The species established in the sowings during heading (DC 52-60) and reached its maximum density during milk development (DC 77-83) in the beginning of July: 2.4 and 2.15 individuals/m<sup>2</sup> for the first and second sowing, respectively. In spring, aphids had low density and no economic importance for the triticale crop. The prevalent species was *Sitobion avenae*, reaching maximum density of 1 to 3.6 individuals per ear for early sowings and 0.2 to 0.7 individuals per year for late sowings at the phenophase of anthesis – milk development (DC 62-79).

The pest fauna of order Heteroptera during the late spring and early summer is represented by 11 species from the families Miridae (5 species), Pentatomidae (4 species) and Scutelleridae (2 species). The species of the genera *Aelia* and *Eurygaster* have greatest economic importance. Their mass development during the years of investigation was observed during anthesis (DC 62-68), peaking at 1.58-0.65 individuals / m<sup>2</sup>, and during dough development and ripening (DC 81-92), peaking at 0.45-2.14 individuals / m<sup>2</sup> for early and late sowings, respectively. The most abundant species were *A. acuminata*, *A. rostrata* and *E. maura* in the early sowings and *A. rostrata* and *E. maura* in the late sowings. The adults and larvae of cereal bugs suck sap from the stems, leaves and ears, but they mainly damage the grains during milk and dough development and ripening (DC 73-92). The grain damages varied from 4.66-7.03% in late sowings to 8.11-9.09% in early sowings.

The insect pests from the other two groups damaging the leaves, ears and stems of triticale were with low densities and had no significant economic importance.

Table 2. Species composition of harmful insects on winter triticale during the growth stages of booting - ripening of the crop (DC 40-92 on Zadoks growth scale)

Group of pests	Order	Family	Species
Sap-sucking pests	Thysanoptera	Phlaeothripidae	<i>Haplothrips tritici</i> (Kurdjumov 1012)
		Thripidae	<i>Limothrips denticornis</i> Haliday 1836
	Hemiptera	Aphididae	<i>Rhopalosiphum padi</i> (Linnaeus 1758)
			<i>Schizaphis graminum</i> (Rondani 1852)
			<i>Sitobion avenae</i> (Fabricius 1775)
		Aphrophoridae	<i>Philaenus spumarius</i> (Linnaeus 1758)
		Cicadellidae	<i>Balclutha punctata</i> (Fabricius 1775)
			<i>B. rhenana</i> Wagner 1939
			<i>Cicadula placida</i> (Horvath 1897)
			<i>Empoasca pteridis</i> (Dahlbom 1850)
			<i>Hardya anatolica</i> Zachvatkin 1946
			<i>Macrosteles laevis</i> (Ribaut 1927)
			<i>Psammotettix alienus</i> (Dahlbom 1850)
			<i>P. provincialis</i> (Ribaut 1925)
			<i>P. striatus</i> (Linnaeus 1758)
<i>Leptopterna dolabrata</i> (Linnaeus 1758)			
Miridae	<i>Lygus rugulipennis</i> Poppius 1911		
	<i>Notostira erratica</i> (Linnaeus 1758)		
	<i>Stenodema virens</i> (Linnaeus 1767)		
Pentatomidae	<i>Trigonotylus caelestialium</i> (Kirkaldy 1902)		
	<i>Aelia acuminata</i> (Linnaeus 1758)		
	<i>A. rostrata</i> Boheman 1852		
	<i>Carpocoris fuscispinus</i> (Boheman 1850)		
	<i>Dolycoris baccarum</i> (Linnaeus 1758)		
Scutelleridae	<i>Eurygaster austriaca</i> (Schrank 1776)		
	<i>E. maura</i> (Linnaeus 1758)		
Diptera	Cecidomyiidae	<i>Sitodiplosis mosellana</i> (Gehin 1857)	
Pests damaging leaves and ears	Orthoptera	Acrididae	<i>Calliptamus italicus</i> (Linnaeus 1758)
		Tettigoniidae	<i>Tettigonia viridissima</i> (Linnaeus 1758)
	Coleoptera	Chrysomelidae	<i>Lema cyanella</i> (Linnaeus 1758)
			<i>Oulema melanopus</i> (Linnaeus 1758)
Hymenoptera	Tenredinidae	<i>Dolerus puncticollis</i> C.G.Thomson 1871	
Stem pests	Hymenoptera	Cephididae	<i>Cephus pygmeus</i> (Linnaeus 1767)

### Conclusion

The insect pest fauna of winter triticale with different sowing dates (last decade of September-October) in 2009-2013 includes 42 species from 6 orders: Orthoptera, Thysanoptera, Hemiptera, Coleoptera, Hymenoptera and Diptera. During early spring (DC 21-39, Zadoks growth scale), the stem pests of order Diptera are most damaging to the sowings. The most important species were *Opomyza florum* and *Phorbia fumigata*. Damages caused by *O. florum* were prevalent in early sowings, while those caused by *P. fumigata* were more pronounced in late triticale sowings. In the phenophases of booting - ripening (DC 40-92, Zadoks growth scale), the most economically important pests were the species from the families Pentatomidae and Scutelleridae, and *Haplothrips tritici*. *A. acuminata*, *A. rostrata* and *E. maura* were the most abundant species in the early sowings

while *A. rostrata* and *E. maura* in the late sowings. There were no significant differences in the peak densities of *H. tritici* adults and larvae in relation to the sowing date.

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**USE OF PHEROMONES FOR MONITORING AND CONTROL  
OF MAIN PESTS OF APPLE IN BULGARIA**

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**Abstract**

The trials were carried out in Bulgaria in the years 2006-2008. The possibilities for reducing the number of treatments with chemical insecticides against pests in apple orchards of Bulgaria, by use of synthetic sex pheromones have been studied. In some species this reduction may be due solely to an adequate monitoring strategy implemented by pheromone traps, indicating the most appropriate time for treatment, thus avoiding inappropriate sprays. The pests that may be successfully controlled using this strategy are leaf miners, apple sawfly, San Jose scale, apple clearwing and leopard moth borer. The key pest of apple – codling moth (CM), *Cydia pomonella* L., which has shown the high resistance to most chemical insecticides used, can be successfully controlled using the method of mating disruption (MD), consisting in disorientation of males, by dispersion of the synthetic pheromone over an orchard lot. A significant reduction of population density of this pest is possible through a combination of mating disruption with application of virus insecticides. The present review paper contains a summary of the selected, most important results of investigations on use of sex pheromones for management of apple pests, carried out by the authors in Bulgaria.

**Keywords:** apple pests, codling moth, sex pheromones, flight monitoring, mating disruption, Bulgaria

**Introduction**

Apple trees are infested in Bulgaria by a large number of pests. Codling moth, *Cydia pomonella* L., is undoubtedly the key pest of apple. The other ones are leaf miners, apple sawfly, San Jose scale, apple blossom weevil, aphids and red spider mite. Recently the population of leaf chewing caterpillars, apple clearwing and leopard moth borer has been also increasing. Unfortunately, most of farmers still follow conventional technologies of plant protection that imply a massive use of various chemical compounds for crop protection. However, conventional methods have shown a number of disadvantages. The great extent of chemicals used leads to a negative impact on the environment as well as on human health, if residues persist on the crop till harvest time. Moreover, repeated use of pesticides induced development of resistant strains of some pests, thus resulting in ineffectiveness of chemical control. In case of codling moth it was documented by Charmillot et al. (1999), followed by Charmillot and Pasquier (2002) in Switzerland and later in Bulgaria (Charmillot et al. 2007). For these reasons in many European countries as well as in the USA and Canada, ecologically approved methods of pest management gained a wide application. It is necessary to introduce these technologies in Bulgarian orchards as soon as possible.

Sex pheromones are harmless and effective means for monitoring and control of pests. Pheromone traps have been widely applied in the world plant protective practice as additional means or substitute to the laborious, traditional methods for monitoring of the insect pest species (Wright 1970, Giannotti and Orlando 1975). Pheromone traps have been also used in Bulgaria for determination of the date of appearance of the most important insect pests of apple and for monitoring their flight dynamics. Pheromones of different origin and traps of different shape have

been tested for monitoring of different pests in our investigations (Kutinkova et al 1997, Kutinkova and Subchev 1998, Kutinkova and Arnaoudov 2001, Kutinkova and Andreev 2001, 2002, 2002a, 2003, Kutinkova et al. 2006, 2006 a, 2008, 2009).

For the key pest of apple, codling moth, the investigations on use of pheromone traps were initiated by Kutinkova (1984). The parameters of traps for this species were established, i.e. the necessary number of traps installed per hectare, height of installation above the ground, and scheme of distribution. For monitoring flight dynamics of codling moth, the traps with pheromone dispensers and sticky bottom, type Pherocon® 1C, Pherocon® VI Delta, all of Trécé Inc. (USA) were mainly used. During the last years black “delta” traps supplied with the capsules of PheroNet Sweden were used. Satisfactory results were obtained changing lures only once per season (Kutinkova et al. 2009).

The first trial on mating disruption (MD) of codling moth in Bulgaria was conducted in the years 1998-1999 at the Experimental Field of the Agricultural University in Plovdiv. Pheromone dispensers of BASF (Germany), i.e. RAK 3+4 were used. Those experiments, provided unsatisfactory results, due to small orchard size, high initial CM population density and missing isolation from migration of codling moths from heavily infested orchards in the neighbourhood (Kutinkova and Andreev 2003).

Isomate-C dispensers, emitting synthetic CM pheromones, were released by Shin-Etsu (Japan) in 1989 and then improved and successfully applied in many countries (Veronelli and Iodice 2004). They were intensely tested by Kutinkova et al. (2009, 2010). At high codling moth pressure, indicated by high initial fruit damage and/or by high hibernating larvae population, granulosis virus products may be helpful as another biological means of control, complementary to mating disruption (Charmillot and Pasquier 2002a). Recently the combination of MD with a virus product, was tested in Bulgaria by Kutinkova et al. (2012).

The most important results of trials on application of pheromones for management of apple pests have been summarized in the present paper.

## **Material and methods**

### **Trials on mating disruption**

Mating disruption, as alternative method for control of codling moth was tested in different regions of Bulgaria. Locations of particular trials as well as area of trial (MD) plots are indicated in the table 2, in the section of RESULTS. For trial plots isolated apple orchards were selected. As reference, served some commercial, conventionally treated orchards, located in the vicinity. Isomate C Plus dispensers, product of Shin Etsu (Japan), each loaded with a minimum of 190 mg of the codling moth pheromone mixture, were applied at the density of 1000 pieces per ha. The dispensers were installed in the upper third of the canopy, before the start of CM flights (at the beginning of April). No insecticides against CM were employed in the trial plots. Occasionally 1-2 treatments of acaricides or aphicides were applied.

Similar, though conventionally treated orchards, located in the vicinity served as reference. In these orchards from 12 to 15 treatments with different chemical pesticides were applied.

### **The trial on MD, combined with application of the virus product for controlling CM**

In an orchard, located at Samuilovo, Sliven District, South-East Bulgaria, the CM pressure was extremely high, as indicated by initial fruit damage (see Table 3). Under these conditions Madex®, the preparation containing the granulosis virus of codling moth (CpGV), product of Andermatt Biocontrol (Switzerland), was applied as complementary measure, ten times per season (at 10-14-day intervals), at a full recommended dosage of 100 ml/ha ( $3 \cdot 10^{12}$  granules/ha) every time. The Madex treatments were combined with fungicide treatments till the end of June, then applied alone, without fungicides, from July on. As in the trials on mating disruption alone, the appropriate reference orchard was used as conventional reference.

### **Monitoring of CM flights by use of pheromone traps**

In the reference as well as in trial plots two triangular traps with a standard capsule (Pheronet OP-72-T1-01), containing 1 mg codlemone were placed every year. The traps were always installed prior to the beginning of flight of CM, then checked twice a week; the caught male moths were

counted and removed. In trial plots catches were scarce or nil. Hence flight dynamics of codling moth in different regions was followed in conventionally treated orchards, serving as reference.

**Evaluation of fruit damage**

The final damage by CM was evaluated by inspection of 3000 or 2000 apples just before harvest or after harvest. The rate of fruit damage was expressed in percentages.

**Evaluation of hibernating population of CM**

In June, corrugated cardboard band traps were placed on the trunks of 40 trees in every trial plot and in every reference orchard, 8 at the border and 32 inside the each plot. They were recovered in autumn, after harvest and the found hibernating CM larvae were counted. The CM population density was expressed as number of hibernating larvae per tree.

**Results and discussion**

**Flight dynamics of codling moth**

It was found that in Bulgaria codling moth (CM), develops at least two full generations that usually overlap each other. Only in the hilly area, at higher altitudes, the second generation may be less numerous. In relatively warmer regions, like South-East Bulgaria, a partial third generation was recorded in the second half of August and in September of the warm years (Fig. 1).

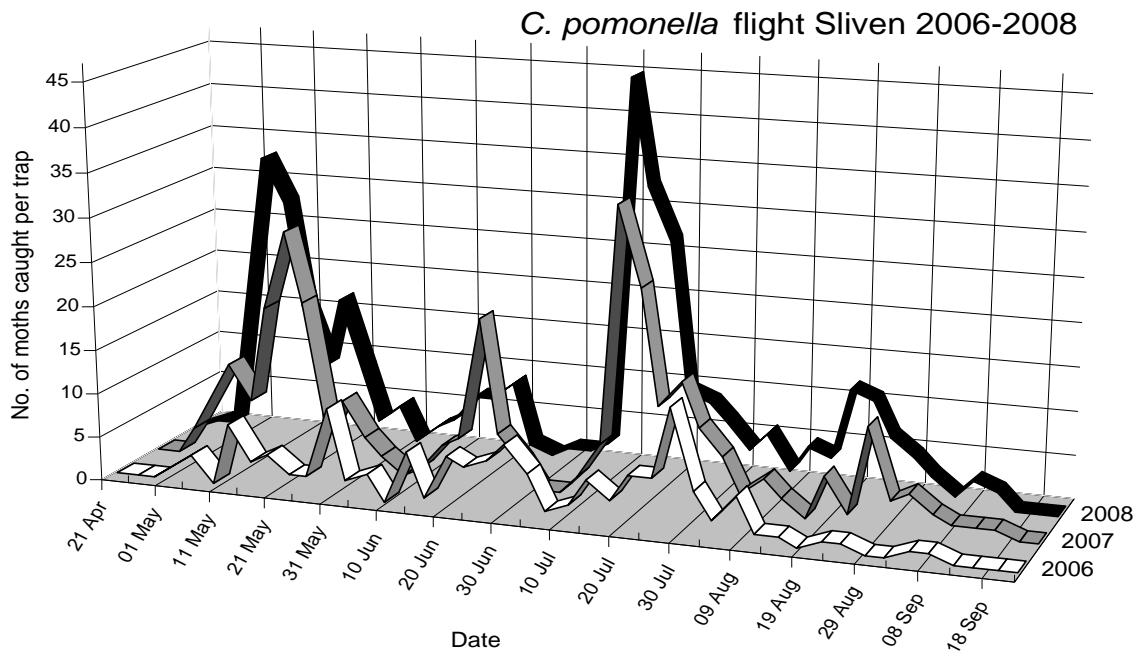


Fig. 1. Dynamics of CM flights in Sliven district, South-East Bulgaria in the years 2006-2008

In the plain land areas of Bulgaria, CM flights usually started at the end of April and lasted with varying intensity till about mid-September; the earliest and the latest flights over the passed years were noted in South-Central Bulgaria, beginning from April 10, (in the Plovdiv district in 2007) and ending as late as on October 3 (in the Stara Zagora district in 2006) – Table 1. It is worth noting that the number of moths caught in the reference orchards, treated with conventional pesticides was, in general, increasing during three years of study.

The results obtained in this study, were confronted with those obtained by Kutinkova (1984) in early 80'ies of the 20<sup>th</sup> century. At that time the CM flights in the Central-South Bulgaria started later and finished earlier; no signs of the third generation was noted. The apparent acceleration and intensification of the development of codling in Bulgaria over the period of more than 30 years may be attributed to the phenomenon of global warming.

Table 1. Dates of the first and last flight and total catches of CM in different regions and different seasons

Region of Bulgaria	Latitude North	Altitude above sea level	Year	Dates of:		Total catches per trap
				first flight	last flight	
Stara Zagora district South-Central	42°32'	~240 m	2006	April 24	October 3	260
			2007	April 20	September 28	288
			2008	April 23	September 16	311
Plovdiv district South-Central	42°22'	~150 m	2006	April 26	September 17	73
			2007	April 10	September 19	145
			2008	April 12	September 15	195
Sliven district South-East	42°40'	~250 m	2006	May 2	September 9	64
			2007	April 30	September 14	141
			2008	April 25	September 14	179
Bourgas district South-East (Pomorie)	42°64'	~30 m	2006	May 3	September 4	40
			2007	April 27	September 18	126
			2008	April 25	September 15	153
Pleven district North-Central	43°25'	~75 m	2007	May 1	September 18	103
			2008	April 24	September 11	82

### Incidence of codling moth in conventional orchards and resistance to pesticides

Under conditions of Bulgaria fruit damage by codling moth, without any control, may exceed 80%. To avoid losses of crop, apple growers routinely apply different insecticides targeted this pest, 12-15 times during the season. Commonly used pesticides against CM are mainly organophosphates and pyrethroids. In spite of numerous treatments, rate of fruit damage by CM was in the studied conventional orchards high and progressively increased in successive years (Fig. 2A). The records of hibernating larvae population in these orchards also indicated at the increasing pressure of the pest (Fig. 2B). Apparently the populations of CM in conventionally treated orchards under study were already resistant to the pesticides used. This interpretation of the noted trends seems reasonable in view of the report of Charmillot et al. (2007), who detected populations of CM resistant to organophosphates and pyrethroids by testing diapausing CM larvae from some apple orchards of South Bulgaria. In that study, efficacy below 20% was found in case of indoxacarb, imidacloprid, azinphos-methyl, phosalone and deltamethrine.



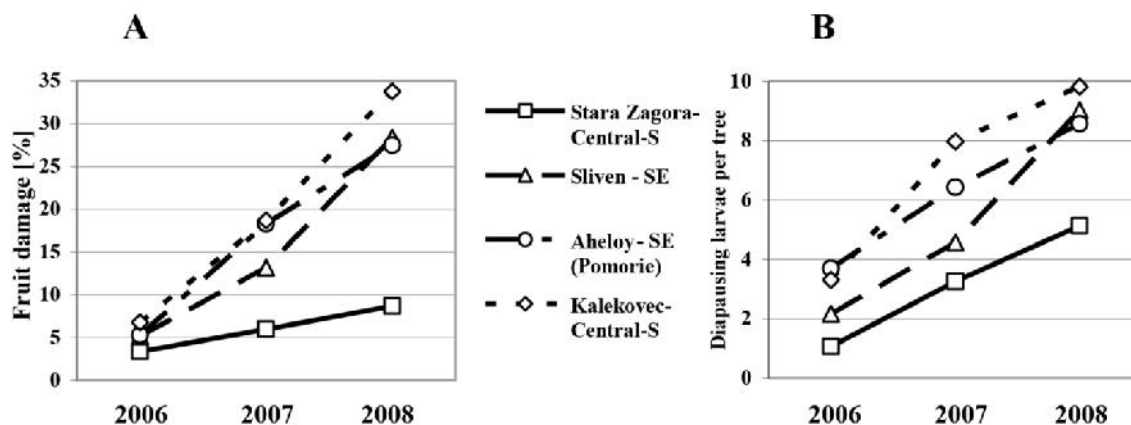


Fig. 2. Fruit damage by CM at harvest (A) and diapausing larvae per tree in corrugated paper bands (B) in four conventionally treated apple orchards in successive years

### Effectiveness of mating disruption in control of codling moth

During the three years of study positive results were obtained in different orchards of the South-Central Bulgaria and South-East Bulgaria. The most important results of these trials are summarized in the Table 2.

Table 2. Results of trials on control of codling moth, by mating disruption, with use of Isomate C plus dispensers applied once per season

Location & altitude	Region of Bulgaria	Trial area [ha]	Initial fruit damage [%]	Year	Fruit damage at harvest [%]		Diapausing larvae per tree in autumn	
					trial	reference	trial	reference
Bogomilovo ~240 m <sup>1,2</sup>	Stara Zagora distr. South-Central	4.0	~2.0	2006	0.06	3.40	0.075	1.075
				2007	2.10 <sup>1</sup>	6.00	1.271	3.263
				2008	1.30	8.70	1.800	5.133
Glufishevo ~145 m <sup>3</sup>	Sliven distr. South-East	1.0	~5.0	2006	0.35	5.20	0.125	2.175
				2007	0.40	13.20	0.225	4.575
				2008	0.70	28.40	0.425	9.025
Malka Vereya ~300 m <sup>4</sup>	Stara Zagora distr. South-Central	1.7	~2.0	2007	0.03	5.20	0.225	3.263
				2008	0.60	5.70	0.325	5.133

<sup>1</sup> The neighbouring pear trees were not treated and were probably a source of CM infestation at the end of the season

<sup>2</sup> Kutinkova et al. 2009a; <sup>3</sup> Kutinkova et al. 2010; <sup>4</sup> Own unpublished data

### Virus products as complementary measures to control codling moth

Trials, carried out for two years in the orchard where initial damage rate exceeded 30% (according to the grower), has shown a high efficiency of mating disruption with Isomate C plus, combined with 10 applications of the virus product Madex<sup>®</sup> (Andermatt Biocontrol AG, Switzerland) per season at the rate of 100 ml per ha. Fruit damage in the trial plot was considerably reduced and the population of diapausing larvae successively decreased. In the reference orchard damage rate and the hibernating CM population dramatically increased, in spite of numerous conventional insecticide treatments.

Table 3. Results of trials on control of codling moth, by mating disruption, with use of Isomate C Plus dispensers, combined with applications of the virus product Madex<sup>®</sup>

Location & altitude	Region of Bulgaria	Trial area [ha]	Initial fruit damage in trial [%]	Year	Fruit damage at harvest [%]		Diapausing larvae per tree	
					trial	reference	trial	reference
Samuilovo 140-160 m <sup>1</sup>	Sliven district South-East	0.8	>30	2006	d.m. <sup>2</sup>	6.1	d.m. <sup>2</sup>	2.16
				2007	2.5	14.8	1.05	4.58
				2008	2.2	28.4	1.65	9.03

<sup>1</sup> Kutinkova et al. 2012; <sup>2</sup> d.m. – data missing for the trial plot

### Conclusions

Considering demand of the market for fruits free of chemical residues and concerns about pollution of the environment, the use of chemical pesticides in Bulgarian fruit production should be reduced or eliminated wherever possible.

Decreasing efficiency of conventional fruit protection – due to development of resistance of key pests to most of the insecticides used – is an additional argument for the search of alternative, non-chemical means of control.

Monitoring of pests may be helpful in reducing the number of treatments, by more precisely timing them.

Mating disruption may be an effective method of pest control, provided that the specific principles are followed: adequate size and shape of an orchard lot, isolation of the orchard as well as proper timing of treatments and right installation of dispensers. Under these conditions Isomate C plus dispensers of Shin-Etsu may be very effective in control of codling moth in apple orchards with a low or moderate population density of CM.

In the orchards with a high initial population density, a combination of MD with application of products containing CM granulosis virus may be a perspective method. Installation of Isomate C dispensers combined with application of Madex<sup>®</sup> leads to decrease of the fruit damage by codling moth. To reduce substantially CM population in that kind of orchards, this method should be applied consequently in successive years.

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**CONTROL OF EARLY BROWN ROT - BLOSSOM BLIGHT IN SOUR CHERRY  
CAUSED BY *MONILIA LAXA***

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**Abstract**

Early brown rot (*Monilia laxa*) in stone fruit species is an economically important fungal disease. Infection and spread occur during flowering. The infected blossoms, young leaves and shoots become necrotic and die. Cool and humid weather creates favourable conditions for early infection causing loss of flowers and great reduction of yield.

The aim of the present study was to follow out the efficiency of different fungicides applied for control of blossom blight caused by *Monilia laxa* at crucial phenological stages of sour cherry development.

Observations on *Monilia laxa* development and spread were carried out in the period 2008-2012, in a sour cherry orchard with three cultivars: ‘Oblachinska’, ‘Schattenmorelle’ and ‘Heimanns Rubin, in the region of Hisar town. In 2008 a treatment with thiophanate-methyl /Topsin M/ at the rate of 150 g/dka was applied at the flowering stage. The infection rate of *Monilia laxa*, reported after flowering, was 32% in ‘Oblachinska’ cv., 17 % in ‘Schattenmorelle’ and 8% in ‘Heimanns Rubin, respectively. In untreated trees of the same cultivars, the infection rate was 97%, 54% and 21%, respectively. In the next years 2009-2012, different fungicides were applied at the white button phenological stage, followed by spraying during flowering stage. After flowering stage, reporting the injuries caused by *Monilia laxa* showed that the infection rate was decreased and in 2012 disease development ceased.

**Key words:** early brown rot, *Monilia laxa* , sour cherry.

**Introduction**

The early brown rot (*Monilia laxa* (Ehrenb.) Sacc.&Vogolino) is an economically important fungicidal disease among sour cherries, which is widespread in Eastern and Western Europe, America and Eastern Asia (Tamm, 1993, 1995; Jones, 1996; Holb, 2008). This pathogen causes burning of the blossoms, necrosis of the petals and the twigs and rotting of the fruits. The initial infection is caused by conidia formed on the mummified fruits and the other infected tissues from the previous year. The critical period for the development of the blossom infection extends from the ‘white bud’ phenophase to the falling of the petals (finish blossoming). The most sensitive phase is the full blossoming phase when all parts of the blossom are susceptible to the infection (Tamm, 1993; 1995; Jones, 1996; Holb, 2008).

The cool, wet or rainy weather during the blossoming of the sour cherries is favourable for the infection of the blossoms and the extent of damages may reach 90% (Szodi, 2008; Obenaus, 2010).

An important factor for the course of the infection is the host plant. The sour cherry varieties Oblachinska and Schattenmorelle are highly sensitive to *Monilia laxa*, while Heimanns Rubin is usually attacked less (Pfeiffer, 2010).

In order to reduce the losses caused by the early brown rot, it is necessary to use chemical substances in the fight against it. Kim (2004) established in vitro that the fungicides delan, polyram, zato, strobi, bayleton, saprol, vektra, skor, topaz and chorus demonstrate high efficiency and inhibit the germination of the conidia of this pathogen.

The prophylactic spraying of copper hydroxide in combination with lime sulphur solution two or three times during the blossoming stage significantly reduces the infection of *Monilia laxa* and increases the yield (Holb, 2005).

Adaskaveg (2005) recommends applying two treatments – during the “white bud” phenophase and the blossoming stage using the fungicides pyraclostrobin, cyprodinil, fenhexamid, fludioxonil, pyrimethanil, dicloran, propiconazole, tebuconazole.

The purpose of this survey is to study the effectiveness of different fungicides used to fight the early brown rot of the type “blossom blight” caused by *Monilia laxa*.

### Materials and methods

The observations on the development and the attack of the fungus *Monilia laxa* were made in a cherry orchard with three varieties of sour cherries: Oblachinska, Schattenmorelle and Heimanns Rubin during the period 2008-2012 in the region of the town of Hisar. The registering of the extent of the attack of the early brown rot of the type “blossom blight” (*M.laxa*) was performed 10 days after the blossoms had run into seeds, when the symptoms of necrosis were visible. We used a sample of 10 inflorescences taken from 5 twigs of 10 randomly chosen trees of each variety and established the percentage of the sick ones.

We observed the phenological development of the sour cherry trees by registering the highly sensitive phases – the beginning, the full blossoming and the end of the blossoming stage. We also observed the weather conditions throughout this period.

The fungicides used over the years have a contact or systemic influence when applied before and during the blossoming stage.

### Results and discussions

On 7<sup>th</sup> April 2008, we conducted a treatment during the blossoming stage using thiophanate-methyl (Topsin-M 70 WP) - 150 g/dka and another treatment after blossoming on 19<sup>th</sup> April – using promicidon (Sumilexe 50 WP) – 150 g/dka (Table 1). The weather conditions (Table 2) were favorable for the accumulation of inoculum of *M. laxa* and the infection during the blossoming stage. The extent of the attack of this disease registered after blossoming was 32% for Oblachinska, 17% for Schattenmorelle and 8% for Heimanns Rubin. For the untreated trees of the same varieties, the extent of the attack of this disease was 97%, 54% and 21%, respectively (Table 3).

Table 1. Fungicidal treatments (2008-2012)

Treatment	2008		2009		2010		2011		2012	
	Data	Active ingredient/ Dose g(ml)/dka	Data	Active ingredient/ Dose g(ml)/dka	Data	Active ingredient/ Dose g(ml)/dka	Data	Active ingredient/ Dose g(ml)/dka	Data	Active ingredient/ Dose g(ml)/dka
Winter-spring	29.02.	Copper hydroxide - 300	16.03.	Copper hydroxide - 300	17.03.	Copper hydroxide - 300	14.03.	Copper hydroxide - 300	20.03.	Copper hydroxide - 300
White bud			10.04.	Mancozeb - 300	08.04.	Thiram - 300	11.04.	Myclobutanil+ Thiram - 30+300	06.04.	Dithianon - 50
Blossom	07.04.	Thiophanate methyl - 150	18.04.	Cyprodinil - 45	15.05.	Thiophanate methyl - 150	15.04.	Thiophanate methyl - 150	12.04.	Flusilazole+c arbendazim - 7,5
After blossom	19.04.	Promicidon - 150	28.04.	Thiophanate methyl - 150	23.04.	Tebuconazole- 75	07.05.	Difenoconazole - 20	23.04.	Thiophanate methyl - 150

In March 2009, when observing the orchard, we found a new generation of conidia of *M. laxa* on the damaged blossoms from the previous vegetation. We also observed secretion of resin from the sick necrotic twigs. These symptoms were most visible on the plants of the Oblachinska variety. The treatments were conducted during the “white bud” phenophase, the blossoming stage and after blossoming. For the first treatment we used the contact fungicide mancozeb (Ditan M-45 WP) – 300 g/dka and the other treatments were conducted using systemic fungicides – cyprodinil (Chorus 50 WG) – 45 g/dka and thiophanate-methyl (Topsin-M 70 WP) - 150 g/dka (Table 1).

Table 2. Meteorological date

Month	Year										Media month average temperature, °C	Month average rains, mm
	2008		2009		2010		2011		2012			
	T	Rains, mm	T	Rains, mm	T	Rains, mm	T	Rains, mm	T	Rains, mm		
March	9.8	12.2	7.3	53.5	6.9	72.4	6.9	74.4	8.4	4.9	6.0	38
April	12.9	35.8	12.1	21.5	12.7	37.9	11.8	18.8	14.4	22.2	12.2	45

- Media day temperature, °C

Table 3. Degree of assault by *Monilia laxa*

Variety	Reporting date, %									
	20.04.2008		05.05.2009		29.04.2010		10.05.2011		30.04.1012	
		Control		Control		Control		Control		Control
Oblachinska	32	97	20	96	21	98	12	87	2	50
Schattenmorelle	17	54	15	47	6	53	5	40	0	22
Heimanns Rubin	8	21	5	26	3	25	3	22	0	10

In March 2009, when observing the orchard, we found a new generation of conidia of *M. laxa* on the damaged blossoms from the previous vegetation. We also observed secretion of resin from the sick necrotic twigs. These symptoms were most visible on the plants of the Oblachinska variety. The treatments were conducted during the “white bud” phenophase, the blossoming stage and after blossoming. For the first treatment we used the contact fungicide mancozeb (Ditan M-45 WP) – 300 g/dka and the other treatments were conducted using systemic fungicides – cyprodinil (Chorus 50 WG) – 45 g/dka and thiophanate-methyl (Topsin-M 70 WP) - 150 g/dka (Table 1).

The weather conditions were favorable for the development of the pathogen and in March the rainfall was above the norm – 53.5 mm (the norm is 38 mm) (Table 2). The extent of the attack of *M. laxa* registered after blossoming was similar to the previous vegetation – 20% for the Oblachinska variety, 15% for Schattenmorelle and 5% for Heimanns Rubin. For the untreated trees of the same varieties, the damages caused by the infection were 96%, 47% and 26%, respectively (Table 3).

The temperature and the rainfall in March and April 2010 (Table 2) were favorable for the accumulation of initial inoculum of *M. laxa*, its development and the course of the infection as well. The spraying was conducted using the contact fungicide thiram (Thiram 80 WG) 300 g/dka during the “white bud” phenophase, the systemic fungicide thiophanate-methyl (Topsin-M 70 WP) - 150 g/dka during the blossoming stage and tebuconazole (Folicur 25 WG) 75 ml/dka (Table 1) after blossoming. For the treated trees, the extent of the attack was low – 6% for the Schattenmorelle, 3% for Heimanns Rubin. The attack of the disease reached 21% for the more sensitive Oblachinska variety (Table 3). As regards the control samples, the high extent of the infection was maintained, reaching almost 100% for the Oblachinska variety.

In 2011, we applied a treatment during the “white bud” phenophase using the contact and systemic fungicide myclobutanil (Sistane super 24 EC) 30 ml/dka + thiram (Thiram 80 WG) 300 g/dka, followed by spraying of systemic preparations during the blossoming stage and after blossoming (Table 1). The combination of the two active substances during the “white bud” phenophase contributed to the reduction of the initial inoculum of *M. laxa* and the elimination of the pathogen in the cherry orchard under observation. The extent of the attack on the different varieties was low and varied from 12 to 3% (Table 3). As regards the untreated trees, the infection was high, ranging from 87 to 22%.

In 2012, the rainfall was scarce – in March it was 4.9 mm (the norm is 38 mm), in April – 22.2 mm (the norm is 45 mm). The applied fungicidal treatments were made during the critical phenophases (Table 4) using suitable preparations (Table 1). This contributed to the cessation of the infection of *M. laxa* as the extent of the attack was 2% for the sensitive Oblachinska variety.

Regarding the untreated trees of Oblachinska variety, the extent of the attack was 50% lower compared with the previous years but the yield was significantly reduced. The damages caused by the disease reached 22% for the Schattenmorelle and 10% for Heimanns Rubin.

Table 4. Fenological observations

Phenophases	Years				
	2008	2009	2010	2011	2012
Green Cone	09.03.	31.03.	27.03.	29.03.	22.03.
White bud	27.03.	10.04.	06.04.	11.04.	05.04.
Beginning of blossom	29.03.	11.04.	09.04.	12.04.	10.04.
Blossom	05.04.	18.04.	13.04.	15.04.	15.04.
End of blossom	10.04.	27.04.	20.04.	30.04.	22.04.

### Conclusions

During the conducted five-year experiment (2008-2012) in a cherry orchard located in the region of the town of Hisar, we established that the fungicidal disease known as early brown rot of the type blossom blight (*M. laxa*) causes significant damages on fruit production. Out of the three monitored varieties, the most sensitive one is Oblachinska, for which the extent of the attack on untreated trees was about 90% and in the year 2012 alone it was 50%. Another variety sensitive to the disease is Schattenmorelle with damages of up to 50% and in the year 2012 alone – 22%. Heimanns Rubin was the variety less attacked by the disease, for which the extent of the attack was about 20% and in the year 2012 – 10%.

The temperature and the rainfall are the crucial factors for the accumulation of a large quantity of initial inoculum of this pathogen and the development of the infection. The inoculation takes place under a temperature of 0-5<sup>0</sup> (Tamm, 1993). During the examined period of five years, the average daily temperatures in March and April were favorable for *M. laxa* – above 7<sup>0</sup> and 12<sup>0</sup>, respectively. Regarding the rainfall, the years 2009, 2010 and 2011 were characterized by a significant quantity of rainfall, while in the year 2012 the rainfall was scarce and the damages of the disease were smaller.

Fungicidal spraying was conducted using contact and systemic preparations. The most effective treatment included threefold spraying of the fungicides during the pre-blossoming stage, during the blossoming stage itself and after blossoming. Under this scheme of combat damage from the disease gradually reduced, as in 2012, its development is stopped.

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**EFFECT OF FERTILIZER RATES ON THE INCIDENCE OF INSECT PESTS OF  
*SORGHUM BICOLOR* (L) MOENCH IN THE NORTHERN REGION OF GHANA**

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**Abstract**

Physiological susceptibility of crops to insect pests may be affected by the form and level of fertilizer used. The study was to identify the insect pests of some newly improved varieties of sorghum and evaluate the effect of different fertilizer rates of inorganic fertilizer on the pests in northern region of Ghana. The experiment was undertaken within the Yendi and Savelugu-Nanton Municipalities of the region in a split plot design with four replicates at each site. Fertilizer levels served as the main plots and varieties as the subplots. Three varieties of sorghum namely, Kapaala (improved variety), Dorado (improved variety) and Kadaga (farmer variety) were used. Three fertilizer rates were also used. These were higher rate (250 kg NPK +250 kg SA/ha) recommended rate (250 kg NPK +125 kg SA/ha) and no fertilizer (as control). Data was collected fortnightly throughout the growing season of the crop and the insect pests and their numbers were recorded. Insects identified on the sorghum crops within both Yendi and Savelugu districts were grasshoppers, stemborers, leafminers, headbugs, midges, spittle bugs and others such as beetles and planthoppers. There were significant differences in the effects of the three fertilizer rates on the incidence of the different insects identified. However considering a particular insect species the fertilizer rates did not show marked differences in their effects. Midges and headbugs highly infested Kapaala and Dorado varieties than the Kadaga variety. The observation was attributed to the relatively compact heads of Kapaala and Dorado varieties which favours oviposition of the insects.

**Keywords:** Sorghum, insect pests, Ghana, fertilizer rates, improved varieties

**Introduction**

*Sorghum bicolor* (L) Moench, traditionally serves as one of the staples in the Savanna zone of West Africa including Ghana. With the best adaptation to the vagaries of variable rainfall and poor soil fertility, sorghum provides the foundation for food security in these rainfed agricultural production systems. There is need to help smallholder farmers increase and secure the production of these staple crops for food and income generation. The development of newly improved varieties in addressing low productivity goes with the optimal environmental conditions and management levels used to maximize the genetic yield potential.

In the developing world, three quarters of all seed planted is derived from stocks maintained on-farm by farmers. Although these stocks provide farmers with a degree of food security, they are susceptible to losses or damage caused by insects (Feldmann *et al.*, 2009).

Biotic stresses like weeds, diseases and pests can be controlled by crop protection products and crop resistance, a practice well established in major crop production areas. Agroecologists contend that links between healthy soils and healthy plants are fundamental to ecologically based pest

management. The control of weeds, diseases and pests could be achieved by keeping the crop strong and healthy by use of chemical substances such as fertilizers.

There is possibility of changing the preference of insects by optimal plant nutritional requirement.

Fertilizers are generally critical for increased agricultural productivity. Studies such as Yardim & Edwards (2003); Jahn, (2004) and John et al., (2004) indicate that pest populations in various agro ecosystems depend on the kind of fertilizers used, the crops grown, and the insect pests present.

Thus the physiological susceptibility of crops may be affected by the form of fertilizer used (Patriquin et al., 1995).

In Ghana research on fertilizer use has mainly been related to yields. Studies on the impact of fertilizer use on the incidence of insect pests are virtually non-existent.

The study sought to identify the insect pests of some newly improved varieties of sorghum and evaluate the effect of different fertility rates of inorganic fertilizer on the pests in the Northern Region of Ghana.

### **Materials and Methods**

#### **Study Area**

The study was carried out in two municipalities of the Northern Region of Ghana. The areas are Yendi(9° 44 8 N, 0° 0 59 W) and Savelugu-Nanton (9° 37 26.4 N, 0° 49 40.8 W) Municipalities. Generally, the Northern Region is much drier than southern areas of Ghana, due to its proximity to the Sahel and the Sahara. The vegetation consists predominantly of grassland, with clusters of drought-resistant trees such as baobabs or acacias. Between May to October is the wet season, with an average annual rainfall of 750 to 1050 mm. The dry season is between November and April. The highest temperatures are reached at the end of the dry season, the lowest in December and January. However, the hot harmattan winds from the Sahara blows frequently between December and the beginning of February. The temperatures can vary between 20°C (59°F) at night and 40°C (104°F) during the day.

#### **Land preparation and sowing**

The land was ploughed, leveled and treated with pre-emergent herbicide. The seeds of the sorghum varieties were planted in the generated randomized plots of 4.5x5m<sup>2</sup> at a spacing of 75x20cm<sup>2</sup>.

#### **Experimental Design and Fertilizer Application**

The experiment was conducted in a split plot design with four replicates at each site. Fertilizer levels served as the main plots and varieties as the subplots. Three varieties of sorghum were used, Kapaala (improved variety), Dorado (improved variety) and Kadaga (farmer variety). Three fertilizer levels were also used. These were higher rate (250 kg NPK +250 kg SA/ha), recommended rate (250 kg NPK +125 kg SA/ha) and no fertilizer as control. The fertilizer was applied as 250kg/ha NPK at planting (for both higher rate and recommended rate) and the second application as 250kg/ha SA (higher rate) and 125kg/ha (recommended rate) at five weeks after planting.

#### **Data Collection**

Data collection started three weeks after planting during the 2012 cropping (wet) season. Data on insect pest population, plant damage and yield were recorded. The data was collected fortnightly throughout the growing season of the crop in a systematic sampling format (Pal and Das Gupta, 1994). 5 plants per row from the 4 middle rows per plot were used and the insect pests found were

identified and their activities, recorded. Un-identified insect pests were also collected and preserved in a specimen bottle containing 98% ethyl alcohol for later identification in the lab or by a Taxonomist. In this study however, since some of the insect pests were nocturnal and would not be observed during the day only the damage symptoms of the pests (and not the individual insect numbers) were taken into account to measure the intensity of the incidence/infestation. The intensity of the incidence was measured by the ratio of the infested plants to the total number of plants in the selected plots of the field at various stages of the growth period. Analysis of variance (ANOVA) was used to analyse the data.

## Results and Discussions

### Insect Pests Identified on the Sorghum Varieties

All three sorghum varieties attracted various insects throughout the growing season. Major insect pests that attacked the crops within the two municipalities were grasshoppers (GH) stemborers (SB), leafminers(LM), spittle bugs(SPB) sorghum midges (SM), headbugs (HB) and others (OT). The others were insects that were found in very minor and isolated cases. These were beetles and planthoppers. Leafminers grazed (mined) the leaf surfaces and also created patterns on the leaf surfaces of the crops. Grasshoppers were found chewing the sorghum leaves and stemborers were identified by deadhearts and the characteristic holes created on the leaf surfaces as a result of the larval feeding. The damage symptoms of leafminers, grasshoppers and stemborers were found throughout the growing period of the crops.

Sorghum midges and headbugs were the most serious pests found on the sorghum during the reproductive stage. In relation to leafminers, stemborers and grasshoppers, sorghum midge and headbugs had relatively lower incidence (Tables 1 and 2). However the effect of their infestations was very severe causing almost 100% yield loss. Several larvae of the midge were found in the glumes which resulted in many chaffy florets as in severe blast infestation (Plate 1b).



Plate 1(a): Normal sorghum head



(b): Chaffy head as a result of Midge infestation

The headbugs on the other hand caused a lot of shriveled grains and mould infestation (Plate 2a&b). Kapaala and Dorado varieties were found to be the most affected, probably due to the fact that they had more compact heads than Kadaga (the farmer variety). This confirms the observation in an earlier study by Tanzubil and (Dekuku1991).



Plate 2 (a): Headbug on developing sorghum grains (b): Shrivelled and mouldy grains as a result of headbug infestation

#### Fertilizer rates versus insect incidence

The insect pests seemed to be exhibiting variable incidence intensity under the three fertility levels. Judging from the LSD (0.05) values, with regards to the different types of insects, there were significant differences observed in the effects of the different fertilizer rates on pests' incidence (Table 1). However for the same insect species, there were no significant differences seen in the effects of the different fertilizer rates on the insect pests' incidence. Nonetheless, the recommended fertilizer rate (RR) seemed to have some effect on headbug (HB) incidence.

#### Varieties versus Incidence

Similar pattern of results described above, was observed under the variety and pest incidence correlation (Table 2).

Leafminers maintained their dominance over the other insects in terms of their incidence on the three sorghum varieties. Headbug incidence was however significantly higher in the two improved varieties (Dorado and Kapaala) than the farmer variety.

Table 1: Effects of fertilizer rates on insect pest incidence on sorghum

Fertilizer Rates	Incidence of Insects (%)						
	GH	LM	SB	HB	SM	SPB	OT
NF	4.22	5.59	4.12	1.68	1.65	2.96	2.88
RR	4.28	5.28	3.89	2.48	1.70	2.50	2.63
HR	4.32	5.24	3.82	1.91	1.73	2.45	3.19
LSD (0.05)	0.66	0.57	0.88	0.82	0.531	1.04	1.28
SED	0.30	0.26	0.40	0.37	0.24	0.47	0.59

Table key: NF=no fertilizer; RR =recommended rate, HR= Higher rate

Table 2: Insect incidence on the three sorghum varieties

Varieties	Incidence of Insects (%)						
	GH	LM	SB	HB	SM	SPB	OT
<b>Dor</b>	4.00	5.37	3.80	2.49	1.78	2.75	3.49
<b>Kad</b>	4.48	5.39	4.20	1.18	1.54	2.58	2.44
<b>Kap</b>	4.35	5.35	3.84	2.40	1.76	2.57	2.78
<b>LSD</b>	0.47	0.48	0.73	0.97	0.63	0.61	0.98
<b>SED</b>	0.23	0.23	0.36	0.48	0.31	0.30	0.48

Table key: Dor=Dorado; Kad =Kadaga, Kap= Kapaala

### Conclusions

Grasshoppers, stemborers, leafminers, headbugs, midges, spittle bugs, sucking bugs and others such as beetles and planthoppers are the most important pests found on *Sorghum bicolor* in the northern region of Ghana.

There were significant differences in the effects of the three fertilizer rates on the incidence of the different insects identified. However considering a particular insect species the fertilizer rates did not show marked differences in their effects. This confirms studies such as Erdal & Edwards (2003); Jahn, (2004) and John et al., (2004) which indicate that pests' responses in various agro ecosystems depend on the kind of fertilizers used, the crops grown, and the insect pests present. Farmers cultivating Kapaala or Dorado varieties should however adopt the higher rate recommendation to minimize headbug infestation.

Although the identified insects attacked all the three varieties of sorghum, midges and headbugs highly infested the two improved varieties than the farmer variety, as a result of their relatively compact heads which favours oviposition of the insects.

Midges and headbugs seem to be real dangers to sorghum production and calls for interventions such as exploring the possibility of using biological control agents as management options.

### Acknowledgement

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**ASSESSMENT OF THE SANITARY STATUS OF POME FRUIT CROPS IN KOSOVO,  
WITH PARTICULAR EMPHASIS TO VIRUS, VIROID AND BACTERIAL DISEASES**

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**Abstract**

Pome fruits represent very important fruit crops in Kosovo, covering around 50% of the total fruit production. In order to understand the phytosanitary status of pome fruits crops in the Kosovo assessment was carried out for detecting 4 viruses (ACLSV, ASGV, ApMV, ASPV), 3 viroids (ADFVd, ASSVd, PBCVd) and 3 bacteria (*Erwinia amylovora*, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *papulans*) on apple and pear. For detection of viruses and viroids serological (ELISA) and molecular techniques (RT-PCR) were used. Concerning bacteria, morphological, biochemical (LOPAT test) and molecular (rep-PCR) tests were performed. This survey showed that ASPV, ACLSV, ASGV and ApMV were detected in the main apple producing areas in Kosovo, while no pear trees were found infected by these viruses. ADFVd was also detected on apple. Moreover, *Erwinia amylovora* was widely distributed on apple and pear in different cultivated areas.

**Key words:** Kosovo, pome fruits, viruses, viroids, bacteria

**Introduction**

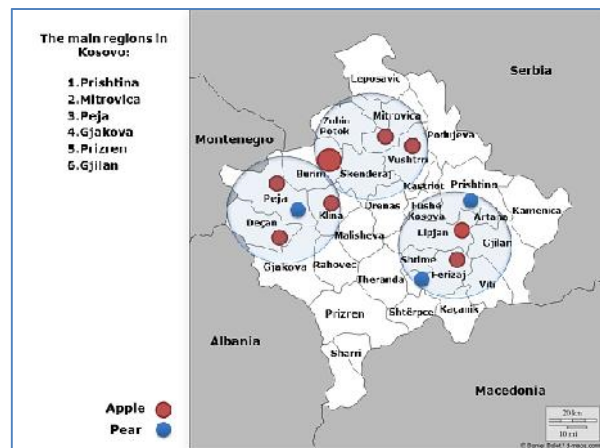
In Kosovo there are good climatic and environmental conditions for cultivating pome fruits. Apple is ranks as the first in the total fruit production in the country; pear is grown to a lesser extent while quince groves are very limited to non-commercial level. In order to maximize their outputs with the least possible expenses, growers were constrained to use their own propagating material. This was accompanied by lack of knowledge on diseases and topped by a shortage of an efficient certification program. Therefore, infected material was distributed and introduced into the country and freely circulated.

To assess the presence and spread of most agents of these graft-transmissible diseases in the field, specific detection techniques were brought to light. For viruses detection combining serological (ELISA) and molecular techniques (RT-PCR) were used, whereas for detection and identification bacterias, Gram reaction, morphological and biochemical tests and molecular assays (PCR and rep-PCR) were performed. .



## Material and Methods

Several visual inspections and field observations were carried out during spring and summer time 2012 for this survey. The sampling from 303 apple and 41 pear trees was performed across the Kosovo territory, covering the main pome fruit producing areas. For the viruses and viroids detection 244 and 60 samples respectively were chosen, while 100 samples were assayed for the bacteria detection. A total of 244 samples were collected, from trees grown in mother plots and commercial orchards located in Prishtina, Lypjan, Mitrovica, Ferizaj, Klina, Burim and Peja municipalities (Fig. 1). Inspected trees varied in age from two to seventeen years old and almost all are imported cultivars. From each of the 219 apple and 25 pear trees three twigs 15 cm in length with leaves were cut, labelled and stored at 4°C for about 3-4 days and then used for further laboratory assays. Visual inspections for specific symptoms of virus, viroid and bacterial infections were also carried out during field surveys. For the bacteria surveys, samples were collected from apple and pear trees grown in mother plots and commercial orchards in Prishtina, Kamenica, Peja, Mitrovica and Ferizaj areas. In Peja and Ferizaj areas, almost all collected samples, showed symptoms which could be associated to the bacterium *Erwinia amylovora* infection.



**Figure 1.** Map of inspected areas related with virus, viroid and bacteria surveys

All investigated samples were tested by DAS-ELISA (Clark and Adams, 1977) for detection of ACLSV, ApMV and ASGV, using commercial kits from Bioreba (Switzerland). Moreover, a total of 60 samples were selected to perform the detection of ASPV virus and viroids. Total RNA was extracted from leaves of pome fruit trees using the silica RNA extraction method (Foissac *et al.*, 2001). Relatively to the detection of Apple stem pitting virus (ASPV) (Menzel *et al.*, 2002) and three viroids (ASSVd, ADFVd and PBCVd) by RT-PCR, according to Di Serio *et al.* (2002) and Lolic *et al.* (2007), cDNA synthesis was performed using 5 µl of viral template, mixed with 1 µl of random hexamers primers (Boehringer Mannheim, GbmH) (0.5 µg/µl), denatured at 95°C for 5 min then immediately cooled in ice.

A 2 hours reverse transcription reaction was carried out at 37°C after adding 4 µl M-MLV (Moloney-Murine Leukemia Virus) buffer 5X (50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM MgCl<sub>2</sub>), 2 µl of 10 mM DTT, 0.5 µl of 10 mM dNTPs, and 200 units M-MLV reverse transcriptase (Invitrogen Laboratories, USA). Inactivation of M-MLV RT was obtained by incubation at 72°C for 7 min. PCR was carried out in a final volume of 25 µl using 2 µl of cDNA, 1 µl of corresponding reverse and forward primers each, 5 µl of GoTaq buffer (5x), 1 µl of dNTPs (10mM each), 1.5 µl MgCl<sub>2</sub> (50 mM). The obtained mix was subjected to thermal cycling at 95°C for 5 min followed by 35 cycles at 94°C for 35 sec, 55°C for 45 sec, and 72°C for 50 sec. The final extension step was at 72°C for 7 min. PCR products in agarose gel 2% were visualized under the UV light.

Relatively to the viroid detection, reverse transcription was performed with cDNA reverse transcription kits according to the manufacturer's recommendations (Applied Biosystems, Foster City USA) and using the total RNA preparations extracted by the method of Foissac *et al.* (2001). The cDNA obtained after reverse transcription was amplified with GoTaq DNA polymerase and the

appropriate primers pair and annealing temperatures. PCR cycling conditions were 95°C for 5 min followed by 30 cycles of denaturation at 94°C for 40 sec, annealing at the appropriate temperature for 40 sec, and extension at 72°C for 50 sec. The final extension step was at 72°C for 7 min. PCR products were separated by electrophoresis in 2% agarose gel and stained with ethidium bromide.

Concerning the bacteria detection, during the winter and spring period, 2012 the asymptomatic samples (bud sticks) were collected, sunken leaf discs in PBS, were taken and put in Petri dishes containing NA and KB agar media. However, during the June, 2012, the symptomatic bud sticks and leaves, were macerated in few drop of SDW in a sterile eppendorf tube using sterile scalpel and forceps, thirty minutes after the maceration, 100µl of macerated tissues were taken from and streaked onto NA and KB agar media using sterile loop. The plates were then incubated at 27°C ± 1°C for 1 week and observed daily for bacterial growth (Jones and Geider, 2001; King, *et al*, 1954). Suspected colonies of *Erwinia amylovora* and *Pseudomonas* spp. were purified and incubated at 27°C ± 1°C. The Kosovan isolates were compared with a collection strains from different countries and hosts, through biochemical tests (Table 1).

**Table 1.** Reference strains of *E. amylovora* and out group strains used for differentiation assays

Strain	Scientific name	Host	Isolation date	Source
Ea I - 204	<i>Erwinia amylovora</i>	<i>Pyruscommunis</i>	2002	Italy
Ea I - 208	<i>Erwinia amylovora</i>	<i>Pyruscommunis</i>	2002	Italy
Ea DZ – 11	<i>Erwinia amylovora</i>	<i>Pyruscommunis</i>	2010	Algeria
CFBP – 1754	<i>Pseudomonas syringaepv.papulans</i> <sup>T</sup>	<i>Malussylvestris</i>	1973	Canada
CFBP – 311	<i>Pseudomonas syringaepv. syringae</i>	<i>Pyruscommunis</i>	1962	France
CFBP-5472	<i>Pseudomonas syringaepv. syringae</i>	<i>Malusdomestica</i>	1988	Canada
CFBP-1670	<i>Pseudomonas savastanoi pv. savastanoi</i> <sup>T</sup>	<i>Oleauropea</i>	-	Ex-Yugoslavia
LMG – 2408	<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	<i>Zantedeschiaaethiopica</i>	1950	UK
CFBP - 1346	<i>Dickeya chrysanthemi</i> biov. <i>chrysanthemi</i>	<i>Chrusanthemummaximus</i>	1969	Italy

\*EaI 204, and EaI 208 were provided by M. Scortichini (CRA, Centro di Ricerca per la Frutticoltura, Rome, Italy),

\*EaDZ (Laalaet *et al.*, 2012); \*LMG, LaboratoriumvoorMicrobiologieUniversiteit Gent, Belgium;

\*CFBP, Collection Française de Bactéries Phytopathogènes, Angers, France.

All colonies obtained were subjected to the LOPAT test (Schaad *et al.*, 2001) for *Pseudomonas* spp. and to the EPPO key tests for the *E. amylovora* identification. Concerning the molecular tests, DNA extraction was performed by using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich-USA), following the manufacturer's recommendations.

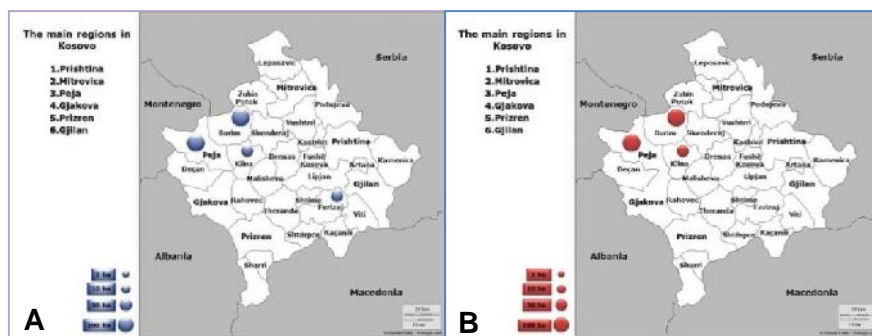
According to Versalovic *et al.* (1991; 1994) and Louws (1996) PCR reactions were carried out in a 25 µl volume for each reaction; the protocol using BOX, ERIC and REP primers was used for the PCR assays. The electrophoretical profile of the agarose gel obtained by rep-PCR using primers sets, respectively, was compared with the reference strains (Table 1).

## Results and Discussion

Twenty four out of 244 samples tested by DAS-ELISA, confirmed to be infected by at least one virus, 12 reacted positively to ACLSV, 11 were positive to ASGV and only 1 sample was reacting positively to ApMV.

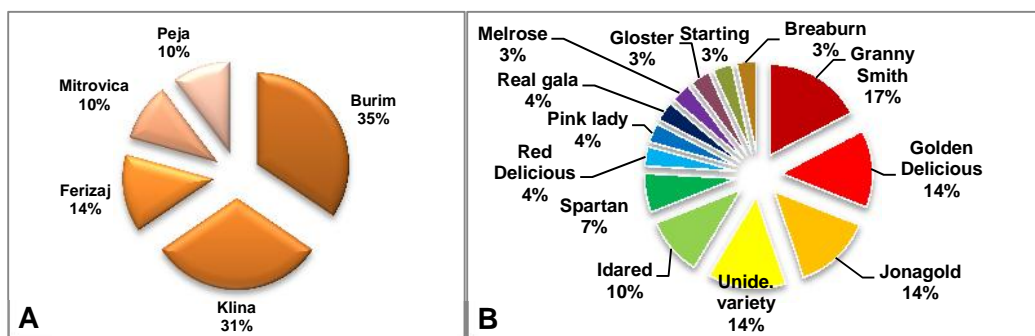
The positive ACLSV trees were recorded in municipalities of Peja, Burim, Klina and Ferizaj, while the ASGV was detected in the municipalities of Peja, Klina and Burim. Peja municipality was the most infected area (Fig. 2).

Among the different cultivars tested, the total ASGV infection was higher on Idared (46%), followed by Red Delicious (36%), whereas the Jonagold and some undetermined apple cultivars presented the same infection rate (9%). Moreover, ApMV was detected only in one Idared cultivar sampled in Peja area.



**Figure 2.** Distribution of ACLSV (A) and ASGV (B) in Kosovo

ASPV was detected in the municipalities Mitrovica, Ferizaj, Klina, Burim and Peja. Burim municipality was the most infected area, followed by Klina, Ferizaj, Mitrovica and Peja (Fig. 3). The highest ASPV infection was found on Granny Smith cultivar (17%) followed by Golden Delicious and Jonagold (14%), while the lower infection was found on newly introduced cultivars Gloster, Starting and Braeburn (3% each of them).



**Figure 3.** Incidence and localisation (A) and distribution of ASPV infected trees among cultivar (B)

The present study shows that ACLSV, ASGV, ApMV and ASPV are widely distributed in commercial orchards where the propagative plant material was imported by some farmers from Serbia (47%), while lower infection rate (6%) was detected on trees which were planted with plant propagative material originated from Albania.

The detection of the viruses was obtained in orchards of different ages however, in general the presence of the viruses was higher (47%) in the older ones (more than 12 years old), and higher was also the infection rate of young infected trees (35%) (up to 5 years old orchards).

The presence of the viruses was similar in the older trees (47%) and the infected young trees (35%). Moreover, bacteria were isolated from symptomless samples collected in Prishtina, Kamenica and Mitrovica, whereas, in isolated bacteria from Peja and Ferizaj areas almost all the trees were showing symptoms which are associated to the Fire blight disease.

After 3 days, the observed colonies in NAS medium were 4-5 mm in diameter, circular, convex and did not show any yellow colour which can be characteristic of *Pantoea* isolates (Janda and Abbott, 2006). Meanwhile, other colonies observed in CCT plates at 27°C were 3-4 mm in diameter, mucoid with shiny surface, semi-transparent and slight violet, characteristically of *E. amylovora*. Relatively to the biochemical characteristics, among the 70 tested isolated, 24 reacted positively to the Levan and the hypersensitivity test on tobacco. The last were subjected to rep-PCR assays, using REP, BOX and ERIC primers, interestingly, 21 isolates showed the pattern which was similar to the reference strains of *E. amylovora* (Table 1). While only one obtained profile was similar to the *P. syringae* pv. *syringae* reference.

### Conclusions

Through a wide survey on pome fruit trees in Kosovo, useful information was obtained on the phytosanitary status of these crops in the country. Most of the sampled trees showed to be infected by at least one of the four viruses ASPV, ACLSV, ASGV and ApMV on apple. No viral infection was revealed from pear. This study evidenced for the first time the presence and real distribution of ACLSV, ASPV, ApMV and ASPV infections in the country. The ASPV showed to be the most prevalent virus in the country with an average rate of 48%, followed by ACLSV and ASGV (5%) and ApMV (1%) respectively. Moreover, this study allowed detecting for the first time on apple, the Apple dimple fruit viroid (ADFVd) reaching an infection rate of 18%. However, no virus and viroid were detected in the Eastern part of Kosovo. Furthermore, *Erwinia amylovora* was extensively distributed on apple and pear in several localities of Kosovo, confirming the wide distribution of this bacteria in the Balkan area. On the other hand, *Pseudomonas syringae* pv. *Syringae* which was detected molecularly in a single apple tree, constitute the first report of this bacteria in the country. Older cultivars ( Idared, Golden Delicious and Red Delicious) showed to be the most infected cultivars comparing to the newly introduced cultivars in Kosovo. The viral and bacterial infections seem to occur in most of the pomefruit growing areas. In addition, viroids were also found in cultivated orchards.

This work will constitute a basic study on the prevalence of pome fruit viral and bacterial diseases in Kosovo and will allow the national authorities to establish a national program to improve the quality of propagative material and the fruit production.

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**MONITORING RESULTS FOR *SCAPHOIDEUS TITANUS* BALL (HEMIPTERA: CICADELLIDAE) IN GRAPE-GROWING REGION OF PODGORICA IN 2012**

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**Abstract**

After the first record of the leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) presence in the vineyards of Montenegro in 2008, in the vicinity of the city of Podgorica, situated in the largest wine-growing region in Montenegro (Podgori ki subregion), during the following years it has mostly spreaded to new localities adjacent to the first detection site.

In the 2012, monitoring was conducted on three localities within the Podgori ki subregion (Šušunja, Lješkopolje and Beri). In all inspected vineyards dominant variety of grapevine was black variety Vranac and, in a lesser extent, black variety Kratošija.

In order to detect presence and the beginning of *S. titanus* nymph emergence, lower side of the oldest grapevine leaves were visually inspected from mid-May to mid-June, while adults were collected from the beginning of July to the end of August. In each locality 10 rows were checked per vineyard. For nymph presence, ten plants in each row were inspected and adults were collected by sweeping with entomological net (10 bits per row). Presence of *S. titanus* eggs were checked in two and three year-old grapevine shoots. In January 2013, in each locality, 50 shoots per vineyard were collected and examined in laboratory.

Results of monitoring showed presence of first nymphal instars during the end of May in localities Šušunja and Beri, and first adults in the second half of July. In both localities population density was low and resulted in 4-9 detected nymphs on the grapevine leaves per vineyard, and 1-10 captured adults. The number of *S. titanus* overwintering eggs, laid on the bark of two and three year-old grapevine wood, were also low in both localities.

Presence of *S. titanus* was not detected in locality Lješkopolje.

**Key words:** *Scaphoideus titanus*, monitoring, grape-growing region, Podgorica

**Introduction**

The leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) is the principal vector for ‘Flavescence dorée’ (FD) phytoplasmas (16SrV subgroups C and D) (Boudon-Padieu, 2003). FD is the most serious phytoplasma disease of the grapevine (*Vitis vinifera* L.) in Europe, and is listed as a quarantine pathogen (Council Directive 2000/29/EC, Annex II/AII). The leafhopper *S. titanus* originates from North America where it lives on wild species of *Vitis* (Vidano, 1966; Maixner et al., 1993). It was introduced in Europe during the 1950s (Bonfils and Schvester 1960). After first introduction it spread from southwestern France over Italy to the Balkans, and to Spain and Portugal (Bertin et al., 2006). The *Scaphoideus titanus* is univoltine species, monophagous on grapevine and overwinters in the egg stage. Eggs are deposited into the bark of two or more years old grapevine branches (Vidano, 1964), rarely in one-year branches. Eggs are laid during the summer by mated females and hatch during the following spring.

The *Scaphoideus titanus* has been observed in countries neighbouring Montenegro: Serbia (Magud and Toševski, 2004), Croatia (Budiniš ak et al., 2005), Bosnia and Hertzegovina (Delic et al., 2007).

Presence of *S. titanus* in Montenegrin vineyards was reported in 2008, in the vicinity of the city of Podgorica, in the largest wine-growing region in Montenegro (Podgori ki subregion). In the following years it has mostly spreaded to new localities adjacent to the first detection site (Radonjic et al., 2012).

In this paper we present results of monitoring for *S. titanus* in 2012 in grape-growing region of Podgorica.

### Materials and Methods

In the 2012, monitoring of *S. titanus* was conducted on three localities in the Podgori ki subregion (Šušunja, Lješkopolje and Beri). In all inspected vineyards dominant variety of grapevine was black variety Vranac and, in a lesser extent, black variety Kratošija.

In order to detect presence and the beginning of nymph emergence, lower side of the oldest grapevine leaves were visually inspected from mid-May to mid-June, while adults were collected from the beginning of July to the end of August. In each locality 10 rows were checked per vineyard. For nymph presence, ten plants in each row were inspected and adults were collected directly from the grapevine plants by sweeping with entomological net (10 bits per row). Collected adults were preserved in 75 % ethanol for subsequent identification. Presence of *S. titanus* eggs were checked in two and three year-old grapevine shoots (approx. 30 cm long) in winter time. In each locality, 50 shoots per vineyard were collected and examined in laboratory.

### Results and discussion

During 2012, nymphs and adults of *S. titanus* were found in two out of three inspected localities. The occurrence of *S. titanus* in grape-growing region of Podgorica is presented in Fig. 1.



Fig. 1. Occurrence of *S. titanus* in grape-growing region of Podgorica (2012)

As a result of visual inspection, the first nymphal instars were observed in localities Šušunja and Beri during the end of May. They were found on the lower side of the oldest grapevine leaves, which were the closest to the bark of the grapevine plant (Fig. 2). During the visual inspections larval skins were also observed on the lower surface of the grapevine leaves. According Aldini et al. (2003) visual inspection of the lower surface of the leaves is better for determining immature stages of *S. titanus*.



a  
b  
Fig. 2. *S. titanus* nymph: a – first instar; b- third instar

(typically two triangular black spots on the dorso-lateral part of the last urite)

According to the number of detected nymphs and sporadically present larval skins, population density of *S. titanus* was ranked as low, and resulted in 4-9 detected nymphs on the grapevine leaves per vineyard. As a result of continuous monitoring in following months, the first adults on these two localities were collected in the third decade of July (Fig. 3). The population rate remained low and number of captured adults varied from 1-10 in July and August (Fig. 4).



Fig. 3. *S. titanus* adult



Fig. 4. Collecting adults by sweeping using entomological net

Results of this survey showed that in 2012, in locality Šušunja low presence and population density of the nymphal stages and adults are similar with data from our previous research (Radonjic et al., 2012). On the other hand in 2012, presence of *S. titanus* was detected the first time in locality Beri. This location was under continuous monitoring from 2010, but presence of the leafhopper was not registered. The latest findings could indicate on gradual spreading of the *S. titanus* in the adjacent parts and localities within the Podgori ki subregion with population size that has been considered as low.

In 2012 presence of *S. titanus* was not detected in locality Lješkopolje, although it was registered in 2011 (Radonjic et al., 2012).



As a result of 150 total examined two and three year-old shoots on presence of *S. titanus* overwintering eggs, they were found in samples taken from localities Beri and Šušunja. Eggs were found laid beneath the bark of shoots (Fig. 5).

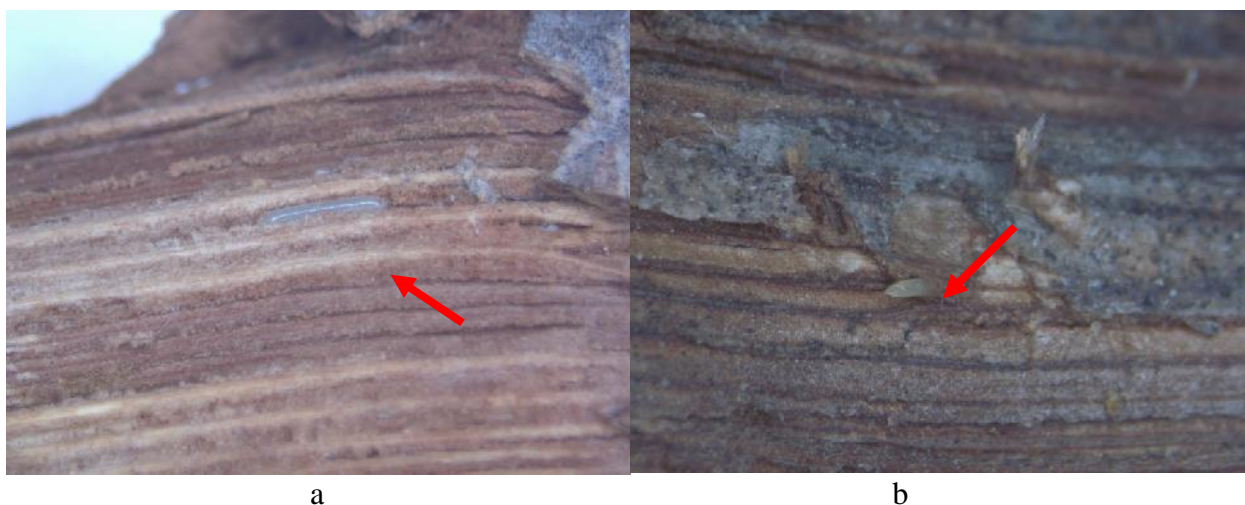


Fig. 5 (a, b). *S. titanus* eggs: laid beneath the bark of two and three year old grapevine shoots

The number of overwintering eggs were also low. It was found 8 and 14 eggs in 50 examined samples taken from Beri and Šušunja, respectively. No eggs presence was found in locality Lješkoplje.

### Conclusion

Data presented here showed that *S. titanus* is established pest in Podgori ki subregion which is the largest wine-growing region in Montenegro. Although population density of the leafhopper was ranked as low in 2012, and similar with previous years, repeated detection of *S. titanus* in locality Šušunja and detection in locality Beri for the first time, indicate its adaptation on agroecological conditions in this wine-growing area and, consequently, real risk to gradually invade the whole this wine-growing area. Established presence of *S. titanus*, as well its spreading, could be serious threaten of possible occurrence of FD phytoplasmas.

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## HAZARD ANALYSIS AND CRITICAL CONTROL POINTS SYSTEM OPTIMIZATION IN A GLUCOSE SYRUP FACTORY

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### Abstract

The glucose syrup is a product obtained from starch slurry through hydrolysis processes. The hazard analysis and critical control points is a management system which aims to assure the safety of the food products by the identification, controlling and prevention of microbiological, chemical and physical hazards. Even if the production process of the glucose syrup is aggressive and unfavourable to the multiplication of microorganisms, the food safety hazards still exist. This paper aims to review the international literature and the general guidelines of food safety assurance in order to optimize the HACCP system already implemented in a glucose syrup factory. Several control and critical control points were identified and for each one the specific monitoring procedure was elaborated. Also, several preliminary programs were identified and centralized in order to prevent the occurrence of hazards.

**Key words:** glucose syrup, HACCP, food safety

### Introduction

Hazard analysis and critical control points system is an “essential element” in the production process of any food product and it should be guided by specific scientific research in order to be efficient for the intended use of the respective product. The glucose syrup is a food product which offers alternative functional properties to sugar as well as economic benefits (Wiley & Sons, 2010). It is obtained through hydrolysis of starch, the process being controlled by measuring the amount of reducing sugars, named dextrose equivalent (DE). Taking into account the DE-value, the glucose syrup can be divided in several types with different properties and applications. The sugar confectionery industry uses glucose syrups with 42 DE and 68 DE, the latter being a direct replacer for inverted sugar syrup (Edwards, 2000). This is the case also for the soft drinks industry, various types of glucose syrups being used in certain “healthy” soft drinks (Mitchell, 1990). The glucose syrup is also used in the fermentation industry as a supplement (D'Amore *et al.*, 1989, Pidocke *et al.*, 2009), in sport drinks to stimulate water absorption (Mettler *et al.*, 2006) and to provide natural energy, in ice cream (Silva Junior & Lannes, 2011) and jams (Javanmard & Endan, 2010).

### Materials and methods

In the international literature there are several research papers which discuss topics such as difficulties and barriers for implementing HACCP system (Ba *et al.*, 2007), factors which affect the food safety management system (Sampers *et al.*, 2012), different tools able to ease the evaluating risk level of hazards (Ryu *et al.*, 2013) and even models of the HACCP implementation in several food industries.

The HACCP study followed the tasks included in the seven principles of the HACCP system described in the second edition of the joint FAO/WHO Food Standards Programme Codex Alimentarius Commission, taking into account the most recent research from literature. The steps, the specific activities and the possible improvements for each of them are presented in Table 1.

**Table 1. Application of the HACCP program in the glucose syrup production process**

<b>Task according to FAO</b>	<b>Activity description</b>	<b>Improvements</b>
Assemble HACCP Team	It is primordial to first establish a multidisciplinary team which can be able to develop an effective HACCP plan.	
Describe Product and Identify Intended Use	The glucose syrup is a product obtained by acid hydrolysis or acid and enzymatic hydrolysis of starch slurry and it is used as raw material in the food industry.	Each product was described in detail in internal product data sheets, taking into account their application in the food industry.
Construct Flow Diagram; On-site Confirmation	Every operation from the flow diagram was analysed considering also the preceding and following steps and verified in the factory.	The flow diagram was completed with the mixing operations and recipients washing.
List all Potential Hazards Conduct a Hazard Analysis Consider Control Measures	The food safety team conducted the hazard analysis by centralizing all the steps mentioned in the diagram flow, the hazards that may be reasonably expected to occur at each step (physical, chemical, microbiological), the gravity, the frequency, the hazard class and the control measurements for each hazard (Chira, 2010).	The specific microbiological hazards presented in Table 3 were taken into consideration, while the chemical and physical ones remained as previously determined.
Determine CCPs	The critical control points were identified using the decision tree presented by FAO/WHO, considering only the steps which were identified to have the risk class 2, 3 or 4 (Chira, 2010).	A previous critical control point, the temperature from the first concentration column, was re-evaluated and transformed into a control point. A new critical control point was identified as the packaging operation and marked on the flow diagram.
Establish a Monitoring System and corrective actions for each CCP	The monitoring system was developed for each CCP by setting the critical limits to be observable and measurable (Chira, 2005). The corrective actions were established.	The monitoring system for the new critical control point, the corrective actions and the responsible person for each action are presented in Table 4.
Establish Verification Procedures, Documentation and Record Keeping	The verification procedures were established using the literature, sampling plans, analysis results, corrective actions in order to demonstrate that the HACCP plan is correctly functioning.	A verification procedure was developed able to establish if the HACCP plan is properly functioning taking into account the analysis results and the records of the passed year.

### Results and discussion

The first and most important step in the development of a HACCP study is the establishing of the HACCP team. The glucose factory from Tandarei has established a multidisciplinary team called “food safety team” which includes only factory employees from seven departments, as follows: production, maintenance, quality control, sales, purchasing, human resources and quality

management. The team members have knowledge and experience regarding the glucose syrups as well as the technology used and they are trained regarding the food safety.

The glucose syrup consists of a mix of several saccharides being characterised by refraction (Brix), dry substance, pH, colour (chromaticity value expressed in ICUMSA units), density and dextrose equivalent. Also, a very important aspect is the carbohydrate composition: glucose, maltose, maltotriose and higher saccharides which give glucose syrups specific application in the food industry. Table 2 presents the sugar composition typical for syrups obtained by acid hydrolysis and by acid-enzyme hydrolysis. In order to be more specific, each product has its own product data sheet in which the specific properties are mentioned, as well as the specific applications and methods of handling.

The factory taken as reference has a HACCP plan which has proved to be efficient until now. However, external auditors and authorities recommended a revision of this plan, in order to take into consideration the effects of changes made in the last year into the factory.

After the new hazard analysis we found that the changes made in the factory did not affect the product from the viewpoint of food safety. Instead, we found that the existent critical control point was insufficient to reduce the danger of microbiological contamination, the real hazard being the cross-contamination. Firstly, by taking into account that in our case the microorganisms are inhibited or even destructed at temperatures ranging from 75 to 82<sup>0</sup>C, as the ones used in the concentration installation, the old CCP was considered redundant and kept only as a CP.

From a hygienic point of view, the hazards from cross-contamination, briefly presented in Table 3, could come from equipment, tanks, storage tanks, packaging and personnel manipulation (Mironescu & Mironescu, 2006). On the other hand, the chemical and physical hazards could appear from the facilities, equipment and also from the personnel. Some of these possible hazards can be eliminated through preliminary programs, but the others only with specific preventing measures.

The updated flow diagram is presented in figure 1 and includes the CCP newly identified. For it we established a monitoring plan presented in Table 4. In order to obtain a fast result, this new CCP will be monitored using a daily test based on ATP detection.

**Table 2. Typical sugar composition for glucose syrups** (source: <http://www.starch.dk>)

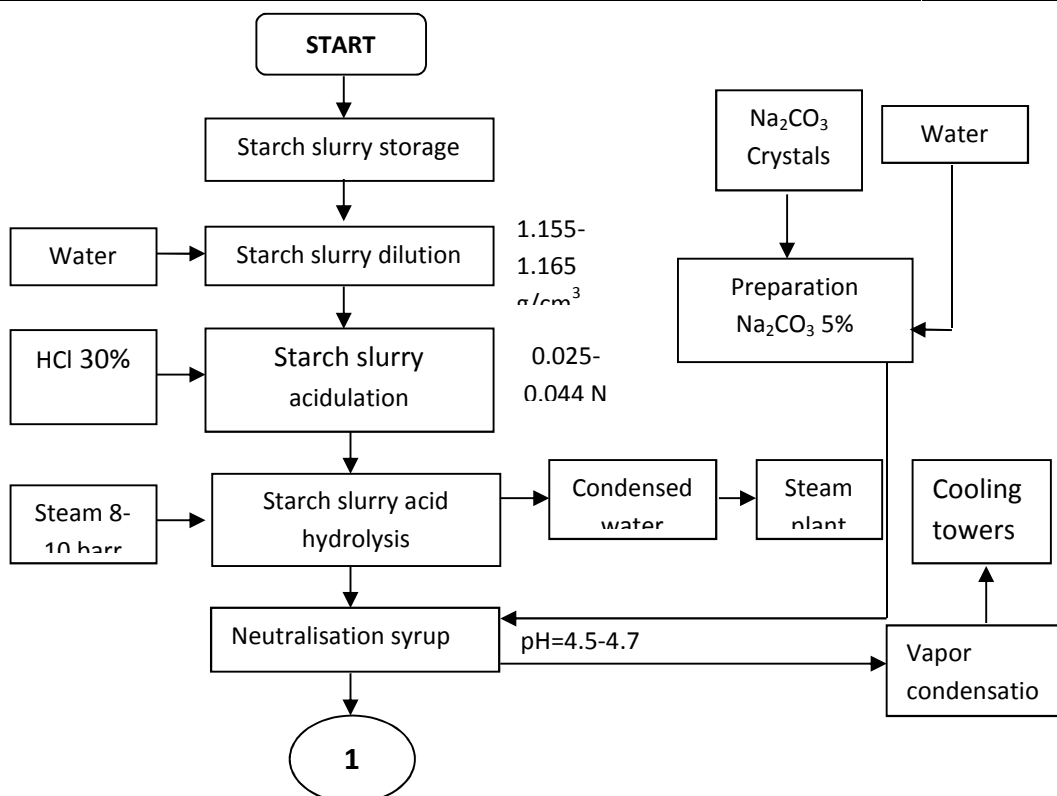
<b>Acid Converted Glucose Syrups</b>			
<b><i>Sugar</i></b>	<b><i>30 DE</i></b>	<b><i>42 DE</i></b>	<b><i>55 DE</i></b>
Dextrose % of DS	10	19	31
Maltose % of DS	9	14	18
Trisaccharides % of DS	10	11	13
Higher sugars % of DS	71	56	48
<b>Acid Enzyme Converted Glucose Syrups</b>			
<b><i>Sugar</i></b>	<b><i>28 DE</i></b>	<b><i>42 DE</i></b>	<b><i>63 DE</i></b>
Dextrose % of DS	5	6	37
Maltose % of DS	8	45	34
Trisaccharides % of DS	16	16	16
Higher sugars % of DS	71	33	13

**Table 3. Microbiological criteria for glucose syrup process** (Mironescu and Mironescu, 2001).

Contamination place	Microorganisms identified
Storage tanks of starch slurry	<i>Fusarium sp.</i> , <i>Absidia sp.</i> , <i>Penicillium glaucum</i> , <i>Aspergillus niger</i> , <i>Lactic bacteria (Lactobacillus)</i>
Storage tanks of carbon-kieselguhr suspension	<i>Escherichia coli</i> , <i>Aspergillus niger</i>
Plastic containers	<i>Penicillium glaucum</i> , <i>Aspergillus niger</i> , <i>Aureobasidium pullulans</i> , <i>Leuconostoc</i> , <i>Bacillus</i>
Walls	<i>Aspergillus niger</i>
Final product (glucose syrup)	<i>Penicillium glaucum</i> , <i>Aspergillus niger</i> , <i>Aureobasidium pullulans</i> , <i>Leuconostoc</i> , <i>Bacillus</i> , <i>Escherichia coli</i>

**Table 4. Monitoring plan for the proposed CCP in the acid and acid-enzymatic glucose syrup production process**

Important hazard	Control measures	Critical limit	Monitoring			Corrective action	Responsible
			Responsible	Method	Frequency		
Moulds and bacteria	Using of food-grade disinfectant detergents	30 RLU	Hygiene responsible	Rapid tests	Once per day	Re-sanitization	Operators



**Fig. 1. Updated flow diagram for the acid and acid-enzymatic glucose syrup** (continued on the next page)

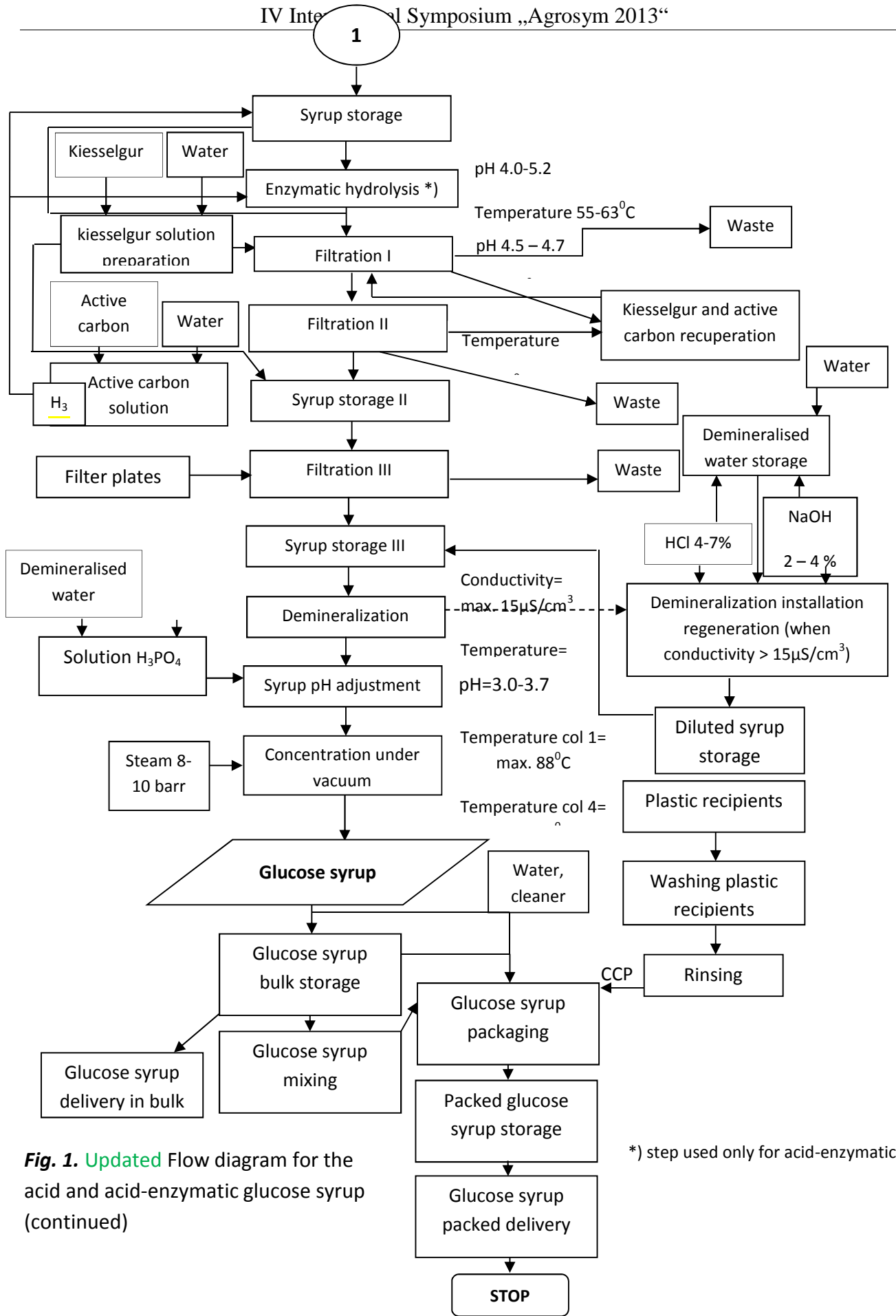


Fig. 1. Updated Flow diagram for the acid and acid-enzymatic glucose syrup (continued)

### Conclusions

The HACCP system already implemented by the factory, although proven as efficient until this moment, was re-evaluated in this study. By reviewing the newly technical and scientific proofs and possibilities it turned out that the current HACCP plan needed some improvements in order to be more effective and to take into account the real hazards, CCP and critical limits.

Although the glucose syrups are used in the food industry only as raw material and they undergo supplementary treatments before becoming a “ready to eat” product, the microbiological hazards still exist and have to be carefully monitored. Even if the production process is not favourable for the growth of microorganisms, the microbiological hazard can appear from cross-contamination and this fact was taken into consideration in this study.

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## LATE BLIGHT ASSESSMENT OF POTATO CULTIVARS USING A NEW EXPRESS METHOD

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### Summary

The proposed assessment method makes it possible to evaluate the level of the foliage/tuber susceptibility of potato cultivars to late blight, caused by *Phytophthora infestans* (Mont.) de Bary, under field and laboratory conditions using a mathematical simulation approach.

**Key words:** *Phytophthora infestans*, late blight, potato resistance

### Introduction

The resistance of potato cultivars to *Phytophthora infestans*, a causal agent of the late blight (LB), still plays a key role in the control of this disease. The use of resistant plants requires no any actions from potato growers during the season; it does not harm to the environment and is usually compatible with other disease management techniques; finally, sometimes such approach is sufficient to reduce the disease development to a tolerant level (Fry, 1982). That is why the assessment of potato cultivars for the LB resistance is an important part of the selection process in the breeding of new cultivars.

There are two known resistance types: vertical (absolute) and horizontal (partial). The first one is race-specific, since it is related to dominant genes (*R* genes) observed in wild *Solanum* species (mainly *S. demissum* and *S. stoloniferum*) used in a potato breeding. *R* genes provide a hypersensitive reaction of infected tissues resulting in the localization of the pathogen penetration site by necrotized tissues. As a result, pathogen perishes, leaving only a small necrotic lesion on a leaf.

Numerous attempts to obtain a long-term resistance using the mentioned *R* genes were unsuccessful because of the development of virulent races that always exist in any *P. infestans* population. As a result, breeders started to use another type of resistance, named partial (horizontal) or field resistance (Turkensteen, 1993; Colon et al., 1995). Unlike the race-specific resistance, this type of resistance only controls the development of the disease and does not suppress it completely. It is usually considered that this type of resistance is polygenic, since it is efficient against all *P. infestans* races and, therefore, has a more stable and prolonged effect, than the race-specific resistance. However, the possibility of genetic recombinations, appeared in “new” *P. infestans* populations due to a sexual process, provided the appearance of more aggressive pathogen strains that caused a gradual decrease of this type of resistance. As a result, the partial resistance of some potato cultivars to various *P. infestans* populations can significantly vary. For example, cv. Sante is considered to be moderately susceptible in Netherlands (Baarveld et al., 2003, p. 23), but susceptible in the Moscow region of Russia (Simakov et al., 2010, p. 75). Due to this fact, there should be a permanent control on the level of the LB infection of cultivated potato varieties.

A common method for the field assessment of the LB resistance of potato is based on the scoring of the foliage destruction; this scoring is usually performed at a certain stage of the plant development.

The LB resistance is also scored under laboratory conditions using artificially inoculated detached potato leaves; the assessed parameter is the size of necroses or the level of sporulation. Results of such assessment are presented using a 9-score scale (Colon et al., 1995). We consider that traditional late blight assessment methods can be improved. To do this, one should use the quantitative value of the LB-caused yield loss, calculated from the dynamics of the dying-off of infected tops during the whole vegetation period, considered as the key assessment factor.

This paper outlines procedures required to realize the above-described idea.

## Materials and methods

### Field tests for foliar blight resistance

The field assessment of the partial LB resistance of tested potato cultivars is carried out against the natural or artificial background via the measurement of the leaf infection level each 10-12 days using a special scale (Table 1).

Table 1. Scale for the assessment of the late blight infection of potato leaves (James, 1971)

Level of infection ( %)	Description
0	- No any signs of infection
0,1	- First single spore-bearing spots.
1,0	- Weak level of infection (5-10 lesions per a plant)
5,0	- About 50 lesions per a plant; 1 of 10 leaf lobes is infected
25,0	- Almost all leaves are infected, but plants still keep a normal form; the field looks green.
50	- Each plant is infected; about 50% of the leaf area is dead; the field looks green with brown spots
75	- The infection is spread over 75% of the leaf area; the field looks brown-and-green
95	- Plants have only single leaves, but the stems are green
100	- All leaves died, and stems are died or dry

Based on this assessment data, one can determine the area under the disease progress curve (AUDPC) in the course of the vegetation season, the corresponding yield losses caused by the early destruction of leaves (%) and the LB resistance level (in scores).

The program for such calculation is available at the website of the All-Russian Research Institute of Phytopathology (Rogozhin and Filippov, 2011; [http://vniif.ru/index.php?option=com\\_content&view=article&id=40&Itemid=30&lang=ru](http://vniif.ru/index.php?option=com_content&view=article&id=40&Itemid=30&lang=ru)).

This program is based on the van der Plank hypothesis (1968), which assumes a direct ratio between the AUDPC for the potato foliage and yield losses. According to our long-term field studies (Gurevich et al., 1977), this dependency can be expressed by the following equation:

$$\tilde{S} = \frac{AUDPC}{q} \cdot 100,$$

where  $\tilde{S}$  is a yield loss (%) caused by an early leaf decay and  $q$  is the number of days between the bud formation phase and the dying-off of non-infected leaves. The average  $q$  value for the early, intermediate, and mid-late potato cultivars is 46, 52, and 84 days, respectively. If the foliage is killed by the frost or desiccant, or the harvesting is carried out before the natural dying-off of the foliage, then  $q$  is considered to be the number of days passed between the bud formation phase and the moment of the foliage death (Rogozhin and Filippov, 2012).

The calculated yield losses are then converted to the scores characterizing the level of the LB resistance in accordance with the 9-score scale, where 9 scores represent the highest resistance level.

#### Detached leaf tests for the foliar blight resistance assessment

The quantitative manifestation of the partial LB resistance within the same potato cultivar depends on the infection load, the level of aggressiveness of *P. infestans* strains and weather conditions. Therefore, an objective assessment can be performed by the arrangement of field trials in regions, which are usually favorable for the LB development (such as the Sakhalin island in Russia or Central Mexico), or under standard laboratory conditions with the use of special tests and the mathematical simulator of the epidemic development (Filippov et.al., 2004).

A laboratory assessment method, developed in the All-Russian Research Institute of Phytopathology (Moscow region, Russia), is based on the joint use of the artificial inoculation of detached potato leaves and the mathematical model, simulating the LB development under standard favorable meteorological conditions and the given primary infection level. This model, based on the measurement of the inoculation efficiency, size of necroses, and sporulation productivity, reproduces the dynamics of the foliage destruction during a vegetation season and calculates the correspondence of this dynamics to the LB-caused yield losses (Gurevich et al., 1979). The method makes it possible to assess a cultivar resistance to the most aggressive *P. infestans* strains, including exotic ones, under isolated laboratory conditions.

The tests are carried out on detached leaves, collected from the tested potato cultivars and inoculated with the studied *P. infestans* isolates, and, in parallel, on detached leaves of the standard potato cultivar, inoculated by the standard *P. infestans* strain.

Plants of the tested cultivars and the standard cultivar (30 plants of each cultivar) are grown under field conditions. During the phase of development of 7-9 leaves, a mid-level leaf is detached from each plant for the testing. Then leaves are transferred to the laboratory facilities and inoculated with the selected pathogen strains. Each tested “cultivar-isolate” pair is compared with the standard pair. The comparison of data, obtained in the course of experiments for each “cultivar-isolate” pair (the number and diameter of necrotic lesions and the sporulation productivity), makes it possible to conclude about any differences in the aggressiveness of isolates from different regions and, therefore, about the level of resistance of tested cultivars.

In the proposed method we used the cv. Sante as a standard cultivar and the N161 *P. infestans* strain, collected in the Moscow region and characterized by a high aggressiveness level and the presence of all 11 basic virulence genes, as a standard strain. The yield losses of the above-mentioned cultivar infected with the chosen strain, make 30% if the weather conditions are favorable for the disease development.

Using the described tests, one can measure the basic parameters of the infection cycle on each tested cultivar as compared to the standard cultivar.

#### Inoculation efficiency test

Ten leaves of each cultivar are inoculated by spraying with the zoosporangial suspension (30000 spores/m<sup>2</sup>); the volume of the suspension is 5 ml per a cuvette. After the inoculation, leaves are incubated in a wet chamber for 3 days at 18° ; then the area of leaves is determined using a photoplanimeter, and the number of necrotic lesions per 1 cm<sup>2</sup> is calculated.

#### Measurement of the size of necrotic lesions

Potato leaves are inoculated with the zoosporangial suspension (1-2 drops per a leaf) using a microdispenser. The concentration of zoospores is the same as for the previous test. Inoculated leaves are incubated in a wet chamber for 18 h at 20° ; then the drops of suspension are removed by a filter paper and the leaves are placed into a wet chamber for additional 3 days. On the 4<sup>th</sup> day, the diameter of necrotic lesions is measured.

#### 3. Sporulation productivity measurement

For this measurement, one can use the leaves from the previous test.

The spore formation intensity is assessed using two methods. One of them represents a visual assessment of the sporulation. The more exact way is to calculate the number of conidia per one lesion using a Goryaev's count number. To do this, one should put 10 leaf lobes with necrotic lesions into a glass beaker and to add 15 ml of distilled water (1.5 ml per a lesion). After the shaking of the beaker, leaves should be removed and the remaining water volume should be measured. The number of conidia per a lesion should be calculated using the Goryaev's chamber.

All after-measurement calculations are performed separately for potato cultivars of three maturing groups. The program developed on the basis of the above-mentioned measurements, calculates the AUDPC value, yield losses, and the level of the late blight resistance of the tested cultivar under constant conditions favorable for the disease development. The program can be found on the above-mentioned web site of the All-Russian Research Institute of Phytopathology.

#### Tuber slice tests for the tuber blight resistance assessment

To assess the LB resistance of potato tubers under laboratory conditions, we propose to use a Lapwood method (Lapwood, 1965, 1967) with some modifications.

Potato tubers are sliced into pieces (7×5×40 mm) in the twenty-fold repeatability. One end of each piece is submerged for 3-5 s into a zoosporangial suspension poured into Petri dishes (2-3-mm layer). After 6 days of incubation, the length of the infected zone is measured by a ruler (mm), and the mycelial covering intensity is determined using a 4-score scale. Tuber slices of the cv. Sante, inoculated with the N161 strain, are used as a standard. According to the expert assessments, the level of the tuber resistance of the cv. Sante to the N161 strain is equal to 5.5 scores of the 9-score scale, where 9 scores correspond to the maximal resistance level.

The cv. Sante and the N161 strain can be replaced by any other “standard” cultivar-isolate pair with the known result of their interaction, expressed in scores. From the practical point of view, it is desirable that the tuber resistance level of the selected “standard” cultivar towards the selected *P. infestans* isolate is scored within the range of 4-7.

Based on the measurements of the size of necrotic lesions and the level of the mycelial covering of tuber slices, the cultivar resistance index is calculated using the following equation:

$$x = \frac{\sum(a \times b)}{n},$$

where  $x$  is the resistance index,  $a$  is the average size of the lesion of the tested cultivar as compared with the standard one,  $b$  is the average mycelial covering intensity as compared with the standard (equal to 1), and  $n$  is the number of slices.

The calculated indices are then converted into scores using a chart (Fig. 1). To determine the LB resistance of tested potato cultivars, one can also use a special program ([http://vniif.ru/index.php?option=com\\_content&view=article&id=40&Itemid=30&lang=ru](http://vniif.ru/index.php?option=com_content&view=article&id=40&Itemid=30&lang=ru)).

### **Results and discussion**

The results, obtained from the laboratory assessment of the LB leaf and tuber resistance of 273 potato cultivars, were processed and added into the corresponding database. According to this study, the majority of cultivars were moderately susceptible and susceptible (93 and 78%, respectively) (Fig. 2). To get an access to the mentioned database, one should use the following link:

<http://vniif.ru/uploads/article/39/> %20 %20 .pdf

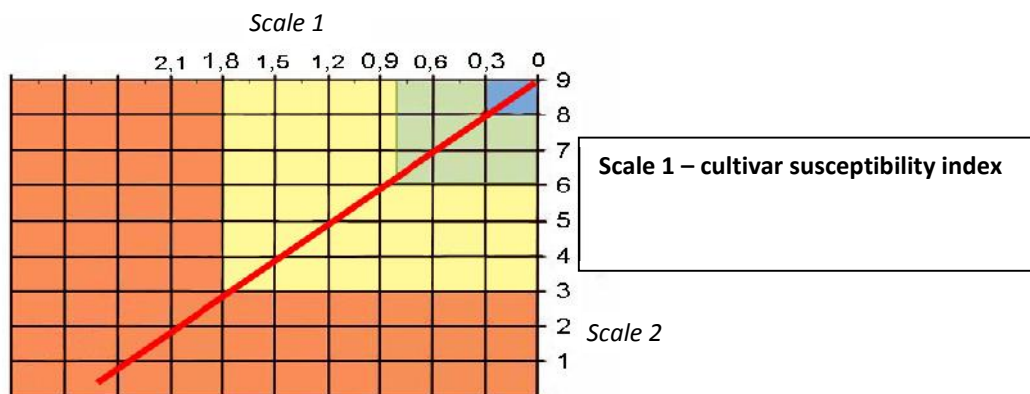


Fig. 1. Chart for the assessment of the LB resistance of potato tubers.

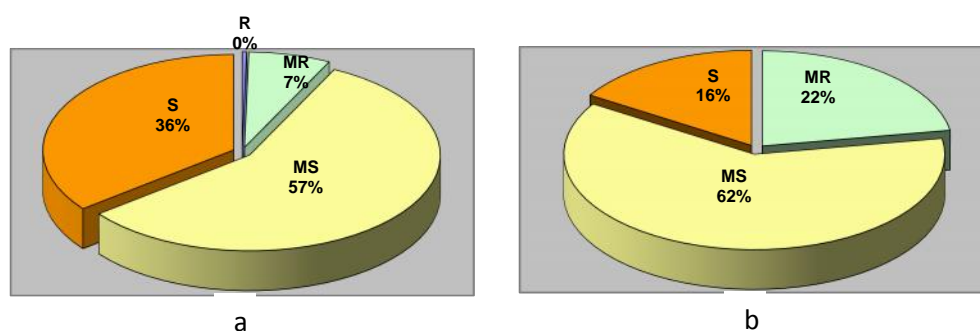


Fig. 2. Results of the laboratory assessment of the LB resistance of (a) potato leaves and (b) potato tubers (2007-2013). Resistance groups: R – resistant (8-9 points), MR – moderately resistant (6-7 points), MS – moderately susceptible (4-5 points), S – susceptible (3 point).

The data on the late blight resistance of potato cultivars added to the above-mentioned database are very important for potato producers. This information allows them to develop the optimal potato protection strategy. For example, the cultivation of the LB-resistant cultivars makes it possible to reduce the number of fungicidal treatments and to reduce the negative influence of the late blight disease on the yield and the quality of tubers; at the same time, the growing of susceptible potato cultivars requires the routine treatment with fungicides to prevent the development of epiphytotes. As we have already mentioned, the common ways to obtain information on the late blight resistance of potato cultivars are the field assessment based on the scoring of a foliage destruction level and laboratory assessment, based on the size of necrotic lesions and the sporulation level measured on detached potato leaves. Both methods have some disadvantages. In the most of regions, weather and phytosanitary conditions, influencing on the results of field trials, vary from year to year, so the field assessment should be repeated for several seasons to obtain reliable results. In the case of laboratory trials, the number of assessed parameters is too small, and their visual estimation is fairly subjective. To overcome these disadvantages, we offer a new method, based on the combination of the artificial inoculation of detached potato leaves and the simulator of a late blight development under standard favorable meteorological conditions and at the given primary infection level. Based on the measurement of the inoculation efficiency, necrotic lesion sizes, and sporulation productivity, the simulator describes a foliage destruction dynamics and calculates its correspondence to yield losses caused by the late blight. The proposed method is able to replace both common methods reducing both cost and time necessary for the cultivar resistance assessment; in addition, it provides the higher assessment accuracy. Now this method is used as a procedure for the state registration of new potato cultivars in Russian Federation.

### Conclusion

The use of the new express method made it possible to assess within a short time period the late blight resistance of all potato cultivars, registered on the territory of Russia, using a severe infection background.

### Acknowledgments

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**CHANGES IN PHENOTYPIC CHARACTERISTICS OF THE MOSCOW  
*PHYTOPHTHORA INFESTANS* POPULATION IN THE PERIOD OF 2000-2011**

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**Abstract**

A long-term monitoring of the Moscow population of *Phytophthora infestans* (Mont.) De Bary, a causal agent of the late blight disease of potato, was performed in the period from 2000 to 2011. A total of 1097 isolates has been assessed for changes in phenotypic characteristics such as the virulence pattern, mating type and metalaxyl resistance. A trend toward an increase in the percentage of the A2 mating type was observed in the first half of the period surveyed. During the whole period, metalaxyl-sensitive isolates remained dominant in the population. In recent years the frequency of the virulence gene 2 began to sharply decrease, whereas the frequency of the gene 10 increased. Among rare virulence genes (genes 5, 6 and 9), the gene 9, which has not been revealed in the Moscow *Ph. infestans* population before 2000, has been stably observed since 2006. Thus, the current *Ph. infestans* population of the Moscow region includes all 11 virulence genes. During the whole period of the study, the *Ph. infestans* population was presented mainly by complex races that include 5-11 virulence genes; the fraction of such complex races makes 50-70%. The most complex race, including all 11 virulence genes, was observed in period 2008 to 2011.

**Key words:** *Phytophthora infestans*, potato, virulence, metalaxyl resistance, mating type

**Introduction**

Potato is one of the most important crops in Russia; the volume of its production makes more than 60% of the total vegetable croppage in Russia (RosBiznesKonsulting, 2012). Being a real „second bread“ for many Russian people, it significantly influences on the food safety of Russia, which, according to the FAOSTAT data, took a second place in the world potato production (about 37 mln. tons/year) up to 2008, when it was outrun by India (FAOSTAT, 2008). In recent years, the annual potato production in Russia made about 33 mln. tons/year (Russia'2012: Statistical pocketbook).

Potato late blight, caused by the oomycete *Phytophthora infestans* (Mont.) De Bary, is the most devastating disease of potato. Leaf blight infection is able to reduce twice a crop productivity during epiphytotic, whereas the tuber blight reduces the storage quality of potato tubers; as a result, total yield losses, caused by this pathogen, can reach 70% (Dyakov and Derevyagina, 2000). Current annual worldwide potato crop losses due to late blight are conservatively estimated at \$6.7 billion; in the case of EU they make about 1 billion euro and include both crop losses and the cost of fungicidal treatments (Havercort, 2008).

During 80's, a sharp increase in the late blight severity was observed in Europe. In the period from 1980 to 1985, the „old“ pathogen population was almost completely replaced by a new one, which included earlier unknown clones (Spielman et al., 1991; Fry et al., 1992) and a „new“ mating type (A2), earlier observed only in the Central Mexico (Fry et al., 1991). New populations became able to the sexual process that increased the population diversity and provided the generation of oospores, able to overwinter on plant debris in the soil. An increased epidemiologic potential of *Ph. infestans* resulted in a sharp decrease in the crop protection efficiency. To develop new efficient late blight control strategies, it is necessary to know the features of the pathogen populations, their

genotypic structure and to forecast possible changes of these parameters in the future.

The purpose of our study was the monitoring of changes occurred in 2000-2011 in the *Ph. infestans* population of the Moscow region, which represents one of the largest potato-growing regions and the largest importer of a potato seed material in Russia. In this study we followed phenotypic characteristics such as the mating type, resistance to metalaxyl-containing fungicides and the virulence pattern.

### Material and methods

*Ph. infestans* isolates were collected during the period from 2000 to 2011 from commercial potato fields and allotment gardens, located in the different sites of the Moscow region. The number of collection sites varied from 5 to 11, depending on the year. During the period of the survey, 1097 *Ph. infestans* isolates were collected in total.

#### Virulence

To study the virulence of isolates, a set of differentiator potato cultivars obtained from the International Potato Center (CIP, Peru) was used. It consisted of 22 genotypes, including all known resistance genes in different combinations. In addition, a test set, containing R<sub>0</sub>-R<sub>11</sub> genotypes and obtained from the Institute of Plant Cultivation and Acclimatization (IHAR, Poland), was used. The virulence pattern assessment was carried out under laboratory conditions using detached potato leaves (Statsyuk et al., 2010).

#### Mating type

The mating type was tested by the growing isolates on rye agar with the known reference strains of the A1 and A2 mating types (Statsyuk et al., 2010).

#### Metalaxyl sensitivity

The sensitivity of isolates to metalaxyl-containing fungicides was determined by the inoculation of fungicide-treated tuber discs with the tested isolates at different fungicide concentrations; depending on the obtained results, isolates were considered as sensitive (S), intermediate (I) or resistant (R) (Cohen and Reuveni, 1983).

### Results and discussion

**Mating type.** The results of the mating type analysis are shown in Fig. 1. For each year, the pathogen population included isolates of both A1 and A2 mating types. In some years the presence of A1A2 isolates, able to form oospores with both A1 and A2 types of isolates, was also revealed. Therefore, the possibility of the sexual process within the pathogen population of the Moscow region remained rather high during the whole surveyed period. The frequency of the „new“ A2 mating type reached the peak value (95%) in 2005 and since then has gradually decreased to 14.3% in 2011.

A gradual increase in the frequency of A2 isolates in the first half of the surveyed period corresponds with the data obtained for the same population in 1997-1998 (Elansky et al., 2001), when the average frequency of the A2 isolates was 28%. Thus, after the first detection of the A2 isolates in Russia in 1985 (Vorobyeva et al., 1991) their frequency in the studied population continued to grow until 2005 and then began to decrease. This trend differs from the situation in the most of European countries ([www.eucablight.org](http://www.eucablight.org)) where the domination of the A2 mating type is recorded in recent years. This can be explained by the appearance of 13\_A2 (also referred to as



„Blue 13“) *Ph. infestans* genotype. It was detected for the first time in 2005 in UK and was characterized by an increased aggressiveness and virulence towards the commonly cultivated commercial crops (Cooke et al., 2007; Lees et al., 2009; White and Shaw, 2009). During next several years this genotype rapidly spread throughout UK, appeared in other EU countries and replaced the most of old genotypes. However, this genotype still has not been revealed in Russia (data not shown).

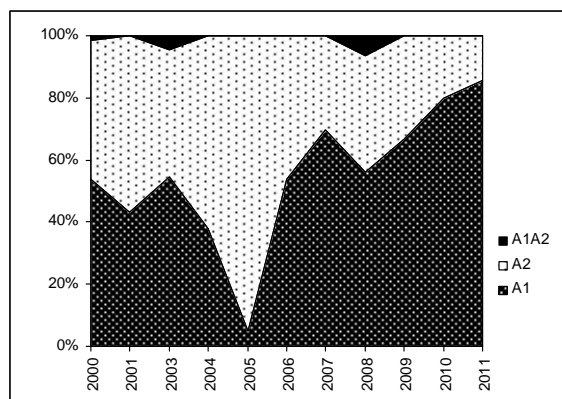


Fig. 1. Dynamics of changes in the ratio of different mating types in the *Ph. infestans* population of the Moscow region in 2000-2011.

**Metalaxyl sensitivity.** The results of the metalaxyl sensitivity monitoring are shown in Fig. 2. During the whole surveyed period, the examined *Ph. infestans* population was represented mainly by metalaxyl-sensitive isolates. This can be explained by the fact that many potato fields in the Moscow region (especially small, private allotment gardens which makes the greater part of the total potato field area of this region) usually remain untreated with fungicides. Therefore, there is no any pressure on the pathogen to maintain its resistance to fungicide on these fields. At the same time, data from some European countries (France and Netherlands) where large commercial potato fields are regularly treated with metalaxyl-containing fungicides shows the domination of resistant isolates in the local pathogen populations ([www.eucablight.org](http://www.eucablight.org)).

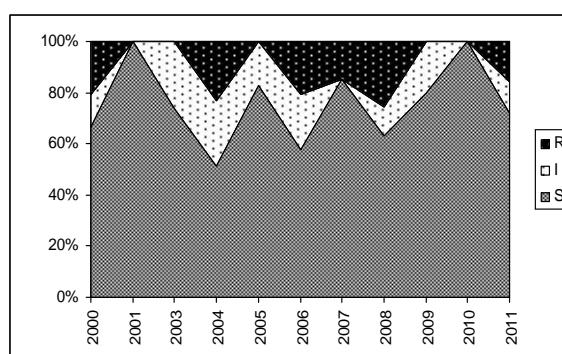
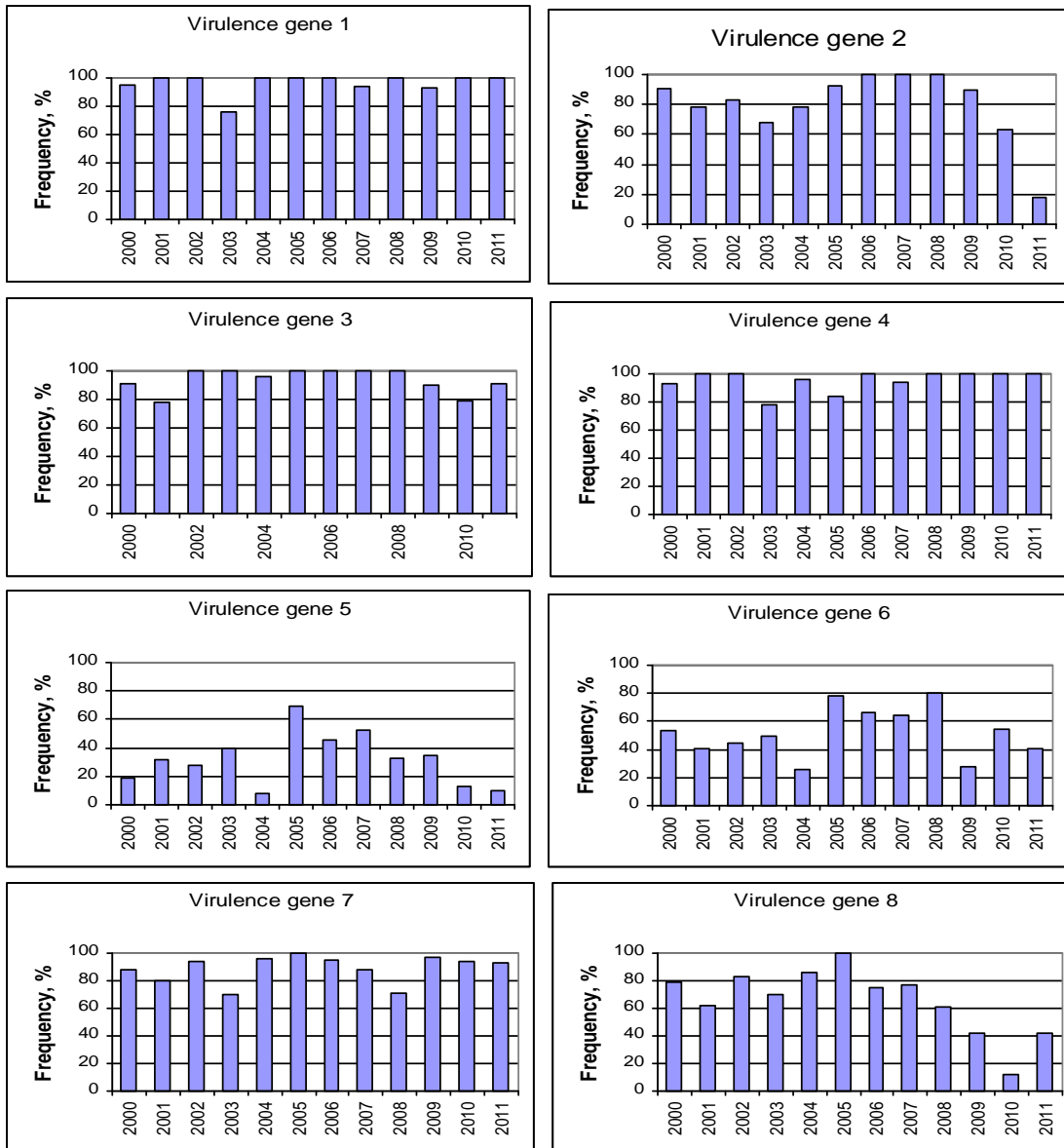


Fig. 2. Dynamics of changes in the metalaxyl sensitivity of the *Ph. infestans* population of the Moscow region in 2000-2011.

**Virulence.** Long-term changes in the frequencies of individual virulence genes in the *Ph. infestans* population of the Moscow region are shown in Fig. 3.



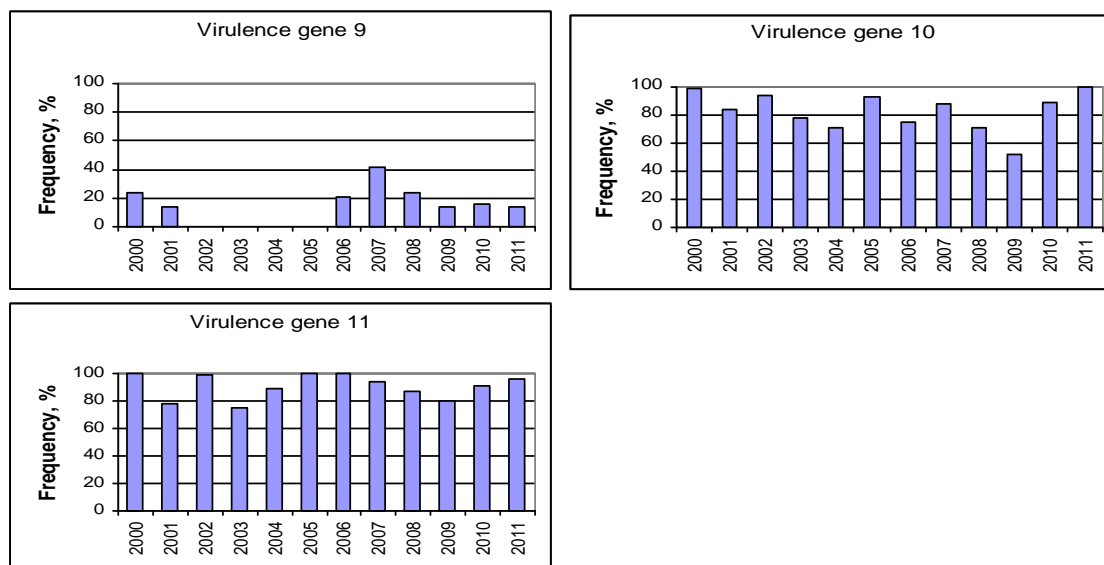


Fig. 3. Changes in the frequencies of individual virulence genes in the Moscow *Ph. infestans* population in 2000-2011.

According to the obtained results, the dynamics of changes in the frequency of individual virulence genes can be described as follows: the frequencies of the virulence genes 1, 3, 4, 7, and 11 remained at the stable level during the whole studied period and varied within the range of 75.4-100%. The frequencies of the genes 10 and 2 remained around the same level as those of the above-mentioned genes. However, in the recent years the frequency of the gene 2 began to sharply decrease, from 100% in 2008 to 17.6% in 2011, whereas the frequency of the gene 10 increased from 51.8% in 2009 to 100% in 2011. The frequency of the gene 8 remained rather high (61.9-100%) until 2005, then began to gradually decrease up to 12% in 2010 and finally increased to 42.2% in 2011.

The group of rare virulence genes includes genes 5, 6, and 9. The frequencies of genes 5 and 6 stably remained at the low (7.5-69.7%) and low-intermediate (26.1-80.2%) levels, respectively. The frequency of the gene 5 gradually decreased since 2005 and reached 9.9% in 2011. The gene 9, which has not been revealed in the Moscow *Ph. infestans* population before 2000, stably presents in the population since 2006, although its frequency remains low (13.4-41.1%).

Results of this survey also showed that the pathogen population was presented mainly by complex races, including from 5 to 11 virulence genes. The fraction of such complex races made 50-70% (data not shown). The most complex race, including all 11 virulence genes, was observed in period from 2008 to 2011.

### Conclusions

The performed analysis allows us to conclude that the Moscow population of *Ph. infestans* still remains very complex and diverse. During the period of the survey, a new virulence gene appeared in the „potato“ subpopulation, and now it includes 11 virulence genes and consists mainly of complex races that provides its high level of aggressiveness. A low percentage of metalaxyl-resistant isolates provides a high efficiency of the treatment of potato plants with metalaxyl-containing fungicides. However, the use of such fungicides should be controlled to prevent the development of the resistance to metalaxyl.

The long-term presence of both A1 and A2 mating types in the population provides a high possibility of a sexual process. This process results in the corresponding increase in the recombination frequencies and, therefore, in the population diversity. In addition, it causes the

generation of oospores, able to overwinter on plant debris in the soil. The possibility of the oospore formation provides an important additional source of infection of potato plants in spring. Therefore, the strict observance of the crop rotation is strongly recommended in the Moscow region; the time interval between potato planting on the same field should not be less than 3-4 years.

### Acknowledgements

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## CORRELATION OF PHENOLIC COMPONENTS IN RED AND PURPLE TOMATOES

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### Abstract

Different parts of plants (roots, leaves, flowers, fruit, stem, bark) have been successfully used to treat numerous diseases. Tomato is known for its medicinal properties. The components that affect its activity are different phenolic compounds.

In this paper, we compared the content of phenolic compounds between the Russian Black Prince variety, type were tested, which is with high content of anthocyanins with hybrid Sidra F<sub>1</sub> selections Institute of Vegetable S.Palanka. In the phase of technological maturity, the selection of sample produce for the purpose of chemical analysis has been performed. The object of the paper has been to define and establish the correlation between the total phenolic compounds and their antioxidant activity in the ethanol extracts of tomato .

**Key words:** phenolic components, antimicrobial properties, tomato, extract.

### Introduction

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities and low toxicity (Vaquero et al., 2010). Antimicrobial activity of herbs has been known and described for several centuries (Begamboula et al., 2003). Many naturally occurring compounds found in edible and medicinal plants, herbs, and spices have been shown to possess antimicrobial functions and could serve as a source of antimicrobial agents against bacteria and fungi (Deans and Ritchie, 1987; Janssen et al., 1985). Several studies have pointed out the possibility to use essential oils and/or their components in medical and plant pathology as well as in the food industry for the control of microorganisms pathogenic to consumers and/or responsible for food spoilage (Cantore et al., 2009). Human organism is exposed to a large number of foreign chemicals everyday . The most of which are man-made and our inability to properly metabolize them negatively affects our health by the generation of free radicals. Free radicals are also generated during normal metabolism of aerobic cells (Carmen and Florin, 2009; Ghaseme et al.,2009). The oxygen consumption inherent in cells growth leads to the generation of series of oxygen free radicals. Highly active free radicals and their uncontrolled production are responsible for numerous pathological processes such as cell tumour (prostate and colon cancers) and coronary heart diseases (Duh and Yet, 1997). Antioxidants can significantly delay or prevent the oxidation of easily oxidizable substances (Cao et al., 1997). Natural antioxidants are classified according to their mechanism of action as chain-breaking antioxidants which scavenge free radicals or inhibit the initiation step or interrupt the propagation step of oxidation of lipid and as preventive antioxidants which slow the rate of oxidation by several actions but do not convert free radicals. However, there have been concerns about synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) because of their possible activity as promoters of carcinogenesis. There is growing interest toward natural antioxidants from herbal sources.

## Materials and methods

### Determination of total phenolic content

Total phenols were estimated according to the Folin-Ciocalteu method. The extract was diluted to the concentration of 1 mg/ml, and aliquots of 0.5 ml were mixed with 2.5 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and 2 ml of NaHCO<sub>3</sub> (7.5 %). After 15 min at 45 °C, the absorbance was measured at 765 nm using a spectrophotometer against a blank sample. Total phenols were determined as gallic acid equivalents (mg GAE/g extract), and the values are presented as means of triplicate analyses.

### Determination of flavonoid content

Total flavonoids were determined according to (Brighente et al., 2007). A total of 0.5 ml of 2 % aluminium chloride (AlCl<sub>3</sub>) in methanol was mixed with the same volume of methanol solution of plant extract. After 1 hour of staying at room temperature, the absorbance was measured at 415 nm in a spectrophotometer against the blank sample. Total flavonoids were determined as rutin equivalents (mg RE/g dry extract), and the values are presented as means of triplicate analyses.

### Determination of total antioxidant capacity

The total antioxidant activity of the vegetable extracts was evaluated by the phosphomolybdenum method (Satyajit et al., 2007). The assay is based on the reduction of Mo (VI) – Mo (V) by antioxidant compounds and subsequent formation of a green phosphate/Mo (V) complex at acid pH. A total of 0.3 ml of sample extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then, the absorbance of the solution was measured at 695 nm using spectrophotometer against the blank after cooling to room temperature. Methanol (0.3 ml) in place of extract was used as the blank. Ascorbic acid (AA) was used as the standard and total antioxidant capacity was expressed as milligrams of ascorbic acid per gram of dry extract.

### HPLC analysis

HPLC analysis was performed by using a liquid chromatograph (Agilent 1200 series), equipped with a diode array detector (DAD), Chemstation Software (Agilent Technologies, USA), a binary pump, an online vacuum degasser, an autosampler and a thermostated column compartment, on an Agilent, Zorbax Eclipse Plus-C18, 1.8 µm, 600 bar, 2.1×50 mm column, at a flow-rate of 0.8 mL/min. Gradient elution was performed by varying the proportion of solvent A (methanol) to solvent B (1% formic acid in water (v/v)) as follows: initial 0-2 min, 100% B; 2-4 min, 100-98% B; 4-6 min, 98-95% B; 6-7 min, 95-73% B; 7-10 min, 75-48% B; 10-12 min 48% B; 12-20 min, 48-40% B. The total running time and post-running time were 21 and 5 min, respectively. The column temperature was 30°C. The injected volume of samples and standards was 5 µL and it was done automatically using autosampler. The spectra were acquired in the range 210–400 nm and chromatograms plotted at 280, 330 and 350 nm with a bandwidth of 4 nm, and with reference wavelength/bandwidth of 500/100 nm.

All standards for HPLC analysis were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MO, USA) and Alfa Aesar (Karlsruhe, Germany). Acetonitrile and phosphoric acid were of HPLC grade (Tedia Company, USA). Ethanol was of analytical grade (Aldrich Chemical Co., Steinheim, Germany). Spectrophotometric measurements were performed using a UV-VIS spectrophotometer MA9523-SPEKOL 211 (ISKRA, Horjul, Slovenia). Plant sample (10.0 g) were extracted by concentration 70%<sub>vol</sub> ethanol or ethanol (100.0 ml) as a solvent. The extraction process

was carried out using ultrasonic bath (Brason and Smith-Kline Company, B-220, (Smith-KlineCompany, USA)) at the room temperature for 1 hours. After filtration, 5 ml of liquid extract was used for extraction yield determination. Solvent was removed by rotary evaporator (Devarot, Elektromedicina, Ljubljana, Slovenija) under vacuum, and was dried at 60°C to the constant mass. Dry extracts were stored in the glass bottles at 4°C to prevent oxidative damage until analysis.

## Results and discussion

Fruits and vegetables contain antioxidant compounds broadly called polyphenols that are known to reduce oxidative stress and prevent chronic diseases (Ames et al., 1993; Diaz et al., 1997). The antioxidant properties of these compounds are responsible for their anticancer, antiviral and antiinflammatory properties (Ames et al., 1995). They can also prevent capillary fragility and platelet aggregation (Benavente- Garcia et al., 1997).

### Determination of antioxidant compounds

As a chemical structure of phenolic compounds is responsible for their antioxidant activity, so measurement of total phenolics content could be related to antioxidant properties of investigated material. Total phenolics content, total flavonoids content and total flavonoids/total phenolics ratio (TF/TP) are presentand in the Table 1. In all tested samples content of total phenolics was higher than the content of total flavonoids. T evaluated by the Folin-Ciocalteu, has been determined by means of spectrophotometric method. Results show that the total phenolic content was higher in the Black Prince (E<sub>1</sub>) (0.0877 ± 0.0001 g GAE/100g sample) than in Sidra (E<sub>2</sub>) (0.0711 ± 0.0001 g GAE/100g sample)

Table 1. Total phenolics, flavonoids, total flavonoid and total phenolics content ratio (TF/TP) and total antioxidant capacity types of vegetable extracts

Type of extract	Total phenolics ( mg GAE g <sup>-1</sup> d. e.)	Flavonoids ( mg RE g <sup>-1</sup> of d.e.)	$\frac{TF}{TP} 100$ [%]	Total antioxidant capacity (µg AA g <sup>-1</sup> d. e.)
Black Prince(E <sub>1</sub> )	87.71±0.0265*	23.18±0.5437	45.99	48.50±1.1872
Sidra(E <sub>2</sub> )	71.19±0.6583	17.43±0.3898	52.93	72.20±0.7255

\*Results are mean values ± SD from three experiment

Content of plant phenolics in vegetables extracts, expressed as mg/g of dry extracts are show in Table 2 and 3. In crude extracts, the following compounds were identified and quantified: gallic acid, protocatehuic acid, caffeic acid, vanillic acid, chlorogenic acid, rosmarinic acid, ferulic acid, sinapic acid, siringic acid, naringenin, miricetin, rutin and quercetin. The dominant components of cultivar Black Prince extracts were chlorogenic acid and rosmarinic acid. In the extract of cultivar Black Prince were less common acids: protocatehuic acid, sinapic acid, vanillic acid. Content of gallic acid, caffeic acid and ferulic acid are lower than 0.1 mg/ g of dry extracts. In cultivar Sidra extract dominant components were gallic (0.369 mg/g) and caffeic acid (0.545 mg/g).



Table 2. Quantitative and qualitative contents of phenolic components in Black Prince

Sample	Component	Content mgg <sup>-1</sup>
Black Prince	Gallic acid	0.086
	Protocatehuic acid	0.343
	Caffeic acid	0.064
	Vanillic acid	0.146
	Chlorogenic acid	0.799
	Ferulic acid	0.072
	Rosmarinic acid	0.648
	Sinapic acid	0.250

Table 3. Quantitative and qualitative contents of phenolic components in Sidra

Sample	Component	content mgg <sup>-1</sup>
Sidra	Gallic acid	0.369
	Caffeic acid	0.545
	Rutin	0.089
	Rosmarinic acid	0.060
	Naringenin	0.044
	Siringic acid	0.045

### Conclusions

Insensitive research for new, unexplored, natural antioxidant and antimicrobial source is very significant and can bring new natural products in pharmaceutical and food industry for their every day battle with reactive oxygen species. Discovering a natural source of antioxidants could be significant and for artificial toxic antioxidants replacement in food industry. The results of this study clearly indicated that vegetable extracts be use as antioxidant and antimicrobial products. Also, they all possess reductive capabilities. They all are adequate source of phenolic and flavoniods compounds, compounds well known as an antioxidants with high antioxidant activity. This study demonstrate good antioxidant and antimicrobial properties of all investigated vegetables extracts prepared of tomato cultivar Black Prince and cultivar Sidra.

### Acknowledgements

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## ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF LETTUCE

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### Abstract

In recent years, lettuce has been increasingly used in human diet throughout the year because of its nutritional and medicinal properties. In this research, lettuce *Lactuca sativa* L. var. *romana* (marula) was used. The content of the antioxidant compounds (phenolic compounds, L-ascorbic acid,  $\beta$ -carotene and lycopene) and the antioxidant activity were determined in ethanolic extracts of the lettuce by means of spectrophotometric methods. A high content of phenolic components provides favourable antioxidant properties found in the examined lettuce. According to the results, the lettuce extract displays the antioxidant activity, with the total antioxidant capacity of  $78.98 \pm 0.25$   $\mu\text{g}$  of ascorbic acid/g and 50% inhibition concentration values of  $26.95 \pm 0.99$   $\mu\text{g}/\text{mL}$  for 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity, and  $98.88 \pm 0.94$   $\mu\text{g}/\text{mL}$  for hydroxyl radical scavenging activity.

The antimicrobial activity of the lettuce extract, was tested with bacteria from clean cultures *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC13883, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC13315, *Proteus mirabilis* ATCC14153, *Bacillus subtilis* ATCC6633, and fungi *Candida albicans* ATCC10231 and *Aspergillus niger* ATCC16404. The antimicrobial activity was determined by microdilution method (MIC). The smallest susceptibility to the ethanolic extract of lettuce was exhibited by the bacteria *Staphylococcus aureus* and *Proteus vulgaris* (MIC=78,125  $\mu\text{g}/\text{ml}$ ), while the other selected bacteria and fungi showed higher susceptibility (MIC=39,1  $\mu\text{g}/\text{ml}$ ).

**Keywords:** lettuce, antioxidant activity, antimicrobial activity

### Introduction

Vegetables and fruits are rich sources of antioxidants such as vitamins A, C and E, carotenoids, polyphenolic components, and flavonoids (Mladenovic et al. 2011), which prevent the attack of free radicals thus reducing the risk of carcinogenic illnesses. Consumption of antioxidants in food via natural sources is good for the prevention of cardiovascular diseases, especially arteriosclerosis (Hu 2000).

Lettuce (*Lactuca sativa* L.), an annual plant, belongs to the family *Asteraceae* and is a very significant leaf vegetable which is primarily consumed fresh as a salad or in salad mixtures with other kinds of fresh vegetables. It is the most popular leaf vegetable and is consumed in increasingly greater amounts owing to the realizations regarding its nutritive values (Dupont et al. 2000), as well as the fact that it is used fresh so that all the ingredients remain intact. Lettuce is used almost throughout the year since there are a number of varieties which are successfully cultivated in early spring, during the summer and winter. In everyday nutrition lettuce is of great significance primarily for the amount of biologically active substances it contains, especially phenolic compounds, ascorbic acid, vitamins A and K, folates and carotenoids (Llorach et al. 2008). The nutritive content varies depending on the lettuce type (Mou 2005). The leaf types (Cos and Cutting)

have a significant amount of ascorbic acid, vitamins A and K, folates, carotenoids and  $\beta$ -carotene, lutein and zeaxanthin. These types are especially rich in vitamin A and  $\beta$ -carotene, the amount of which is approximately 15 times larger than the one in the head-forming types of lettuce Crisphead type (Cooper 2004).

Recent research has shown that the ethanol extracts of lettuce possess an antimicrobial activity towards certain strains of microorganisms (Pavlovic et al. 2011).

In this paper the research has been performed on the lettuce *var. romana* (marula). A characteristic of this lettuce type is that it grows upright, has thick, firm, tasty leaves forming a shape of heart. It grows slower than butterhead and crisphead lettuce types. It succeeds better in cold conditions, on wet ground rich in humus. It is more resistant to frost than most lettuce types, so that certain strains are cultivated in winter in the open.

## Materials and Methods

### Sample preparation

Green leaves of the lettuce were extracted by 96% ethanol in the process of cold maceration. The ethanol was removed by a rotary evaporator (Devarot, Elektromedicina, Ljubljana, Slovenija) under a vacuum and was dried at 40°C. The dried extracts were stored in glass bottles at 4 °C to prevent oxidative damage until the analysis.

### Spectrophotometric methods

Total phenols in the lettuce ethanol extracts were estimated according to the Folin–Ciocalteu method (Singleton et al. 1999). The absorbance was measured at 765 nm with a spectrophotometer against a blank sample. Gallic acid (GA) was used to calculate the standard curve. The assays were performed in triplicate; the results were the mean values  $\pm$  standard deviations and expressed as milligrams of gallic acid equivalents per gram of dry extract (mg of GA/g).

The aluminium chloride colorimetric method (Brighente et al. 2007) was used to measure the flavonoids content of the lettuce extracts. Rutin was used as a standard for the calibration curve. The estimation of the total flavonoids was performed in triplicate. The results were the mean values  $\pm$  standard deviations and expressed as rutin equivalents (mg of RU/g of dry extract).

The total antioxidant capacity of the examined lettuce extract was evaluated by the phosphomolybdenum method (Prieto et al. 1999). The assay is based on the reduction of Mo(VI) to Mo(V) by antioxidant compounds and subsequent formation of a green phosphate/Mo(V) complex at acid pH. Ascorbic acid (AA) was used as the standard, and the total antioxidant capacity was expressed as micrograms of AA per gram of dry extract ( $\mu$ g AA/g dry extract).

The capacity to scavenge the “stable” free radical DPPH was monitored according to the method of Takao *et al.* (1994) adopted with suitable modifications from Kumarasamy *et al.* (2007). The 50% inhibition concentration (IC<sub>50</sub>) value, defined as the concentration of the test material that leads to 50% reduction of the free radical concentration, was calculated as micrograms per millilitre through a sigmoidal dose-response curve.

The ability of the examined lettuce to inhibit a non-site specific hydroxyl radical-mediated peroxidation was carried out according to the method described by Hinneburg *et al.* (2006). The percentage inhibition values were calculated from the absorbance of the control and of the sample, where the controls contained all the reaction reagents except the extract or positive control substance.

Ascorbic acid was determined according to the method of Klein and Perry (1982). The content of ascorbic acid was calculated on the basis of the calibration curve of standard L-ascorbic acid (0.020–0.12 mg/ml). The results were expressed as milligrams of ascorbic acid/100 g of fresh lettuce.

-Carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The absorbance was measured at 453, 505, 645 and 663 nm. Contents of -carotene and lycopene were calculated according to the following equations:

$$\text{lycopene (mg/100 ml)} = -0.0458 A_{663} + 0,204 A_{645} + 0.372 A_{505} - 0.0806 A_{453};$$

$$\text{-carotene (mg/100 ml)} = 0.216 A_{663} - 1,22 A_{645} - 0.304 A_{505} + 0.452 A_{453}.$$

#### Microdilution Method ( MIC )

The minimum inhibitory concentrations (MIC) of the lettuce extract against the tested bacteria and fungi were determined using a microdilution method in 96 multi-well microtiter plates (Satyajit et al. 2007). All tests were performed in Muller–Hinton broth (MHB), with the exception of yeast, in which case Sabouraud dextrose broth was used. Growing conditions and media sterility have been checked for each strain. A standard antibiotic, “Amracin,” has been used for controlling the sensitivity of the examined bacteria, while “Nystatin“ has been used as a control for the tested fungi. As an indicator the solution resazurin has been used, and it has been added in each well. After the incubation (on 37 °C for 24h) the minimal inhibiting concentration has been determined visually, on the basis of colour. Each change of colour from purple to pink or clear has been considered to be positive. The lowest concentration that caused the changing of colour has been taken as MIC value. Material has been measured three times and the calculated mean value has been taken for MIC.

### Results and Discussion

The results of the analysis of the antioxidant compounds content and the antioxidant activity of *Lactuca sativa* L. var. *Romana* extract are given in Table 1. The obtained values show that the ethanolic extract of the examined lettuce contains a high concentration of the total phenols and flavonoids. Owing to the presence of these compounds the antioxidant capacity is somewhat higher than in the earlier examined varieties of lettuce (Zdravkovic J. et al. 2013). Phenolic compounds and flavonoids have been reported to be associated with the antioxidant action in biological systems, mainly due to their reduction–oxidation properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Saha et al. 2008). However, the ethanolic extract of lettuce *Romana*  $IC_{50} = 26,95 \pm 0,99$   $\mu\text{g/ml}$  showed less antioxidant activity of the ethanolic extract of, for instance, AA  $IC_{50} = 10.61\mu\text{g/ml}$  and BHT  $39.25 \mu\text{g/ml}$ . We could say that the amount of the other antioxidant compounds (L-ascorbic acid,  $\beta$ -carotene and lycopene) remains within the boundaries recorded in other lettuce types (Llorach et al. 2008).

Table 1. The Content of the Antioxidant Compounds and the Antioxidant Activity of the tested *Lactuca sativa* L. var *Romana* Extract

Compound	Value
Total phenolics (mg of GA/g)	79,54 ± 0,43
Flavonoids (mg of RU/g)	27,98 ± 0,88
Total antioxidant capacity (µg of AA/g)	78,98 ± 0,25
DPPH, IC <sub>50</sub> (µg /ml)	26,95 ± 0,99
HRSA, IC <sub>50</sub> (µg /ml)	98,88 ± 0,94
L-ascorbic acid (mg /100 g)	6,80 ± 0,35
-carotene (mg /100 g)	3,40 ± 0,28
lycopene (mg /100 g)	0,40 ± 0,03

GA, gallic acid; RU, rutin, AA, ascorbic acid;

DPPH, 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity;

HRSA, hydroxyl radical scavenging activity.

Table 2. Minimum Inhibitory Concentrations of the Ethanolic Extract of *Lactuca sativa* L. var. *Romana* and the Standard Drugs for Eight Indicator Strains

Microbial strains	MIC (µg /ml)		
	<i>Romana</i> ethanolic extract	Amracin	Nystatin
<i>S. aureus</i> ATCC 25923	78,12	0,97	/
<i>K. pneumoniae</i> ATCC 13883	39,10	0,49	/
<i>E. coli</i> ATCC 25922	39,10	0,97	/
<i>P. vulgaris</i> ATCC 13315	78,12	0,49	/
<i>P. mirabilis</i> ATCC 14153	39,10	0,49	/
<i>B. subtilis</i> ATCC 6633	39,10	0,24	/
<i>C. albicans</i> ATCC 10231	39,10	/	1,95
<i>A. niger</i> ATCC 16404	39,10	/	0,97

The results of the analysis of the antimicrobial activity obtained by the dilution method are given in Table 2; MICs were determined for eight selected indicator strains. The results presented in Table 2 reveal antimicrobial activity of the ethanolic extract of *Lactuca sativa* L. var *Romana* within the concentration range of 39,10 µg/ml to 78,12 µg/ml. Equal susceptibility to the ethanolic extract of the lettuce among the strains tested was exhibited by *K. pneumoniae* ATCC 13883, *E. coli* ATCC 25922, *P. mirabilis* ATCC 14153, *B. subtilis* ATCC 6633, *C. albicans* ATCC 10231 and *A. niger*

ATCC 16404 (MIC= 39,10 µg/mL), and somewhat lower by *S. aureus* ATCC 25923 and *P. vulgaris* ATCC 13315 (MIC= 78,12 µg/mL).

### Conclusion

On the basis of the obtained results, it can be concluded that the lettuce *Lactuca sativa* L. var *Romana* displays a pronounced antioxidant activity owing to the presence of the antioxidant compounds. The amount of the antioxidant components (phenolic compounds, L-ascorbic acid, β-carotene and lycopene) lies within the boundaries recorded in other lettuce types. The ethanolic extract of the examined lettuce showed a favourable antimicrobial activity when applied *in vitro* against the tested bacteria and fungi. These experiments confirmed that lettuce is necessary in human diet as a source of antioxidant components and vitamins, especially since it is used raw so that its nutritive value remains preserved. By comparing it to other lettuce types, it has been concluded that the amount of the examined antioxidant components depends on the genotype of lettuce, which gives an opportunity to selectors to work on the improvement of certain characteristics of new lettuce genotypes.

### Acknowledgements

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## SENSORY AND CHEMICAL EVALUATION OF PLUM, APRICOT AND PEAR DISTILLATES

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### Abstract

This study was conducted to evaluate the sensory properties and certain parameters of the chemical composition of twelve samples of alcoholic beverages. The test samples included: six samples of plum distillates produced in different years (1985., 1997., 2000., 2004., 2007 and 2010), three samples of apricot distillates and three samples of pear distillates produced in the years 2004., 2007. and 2010. All samples were collected in the region of Bakač. The sensory properties analysed were: colour, smell, taste, clarity and typicality. The following chemical characteristics were evaluated: ethanol content, total acidity, volatile acids, nonvolatile acids and esters. The quality of the distillates analysed depended both on the type of fruit used to obtain the distillate and year. The analyses showed that the 1985 plum distillate exhibited the best sensory properties (total score 19.30) as well as the highest values for both the alcohol content (55% v/v) and total acidity (2.76 g/l). Volatile acids, nonvolatile acids and esters in the sample were found at 1.992 g/l, 0.768 g/l and 1520 mg/l, respectively.

**Key words:** alcoholic beverages, chemical composition, sensory properties

### Introduction

The Rulebook on the Quality of Alcoholic Beverages (Official Gazette of Serbia and Montenegro, issue 24/) includes a provision stipulating that alcoholic beverages include brandies made from fruits, grapes, edible forest fruits, cereals and other agricultural raw materials, spirits and liqueurs exhibiting specific sensory characteristic coming from the raw materials and additives used and having an alcohol content within the range of 15% v/v to 55% v/v, and fruit wines having an alcohol content of 5-15% v/v.

Fruit brandies are alcoholic beverages obtained by distillation from fermented pomace, mash or juice of fruit, with an alcohol content of at least 25% v/v, unless otherwise stipulated by the Rulebook for certain types of fruit brandies. Fruit brandies must be qualified by the name of the fruit from which they derived. Fruit brandies are marketed in bottles made of glass or other suitable material. The label on fruit brandies must report a statement of the ethanol content. A fruit brandy distillate must not contain more than 86% v/v ethanol. Brandy is an alcoholic beverage with a very long tradition in Serbia and produced by distillation of fermented fruits (Pecic et al., 2013).

Fruit brandies placed on the market must satisfy the following requirements:

- must be clear, colourless or having a colour typical of the particular type of brandy;
- the odor and taste must be characteristic of the particular type of brandy;
- must not have an unpleasant odor and taste;
- must not contain strength enhancers (pepper, paprika, and the like), free mineral acids, nonvolatile organic acids in amounts exceeding the levels considered normal for particular type of brandy and other substances prohibited by the Rulebook.

The Serbian word for brandy "rakija" is of Arabic origin. It derives from the word "al-rak", loosely translated as "sweat". The word arrived in this region with the Turks in the 14th and 15th centuries.

Initially, it referred to the arak beverage produced in Indonesia, Malaysia and other countries in the Far East. Arak was obtained by distillation of the fermented juice or wine made from special types of palm trees. The 15th century marked the beginning of the production of “national drinks” in European countries, i.e. gin in England, schnapps in Germany, aquavit in Scandinavia, vodka in Russia and Poland, and rakija (brandy) in the Balkan Peninsula. At first, they were used not only for therapeutic and medical purposes but also as mood enhancers. In the regions of Serbia, brandy was obtained from different fruit crops, mostly plums. Brandy production in Serbia did not start until the end of the 19th century, after the phylloxera epidemic had destroyed vineyards and reduced wine production.

Major plum cultivars used for brandy production include 'Požega a', 'Crvena Ranka', 'Metlaš', 'Trnošljiva', 'Myrobalan', ' a anska Rodna', ' a anska Lepotica'. These cultivars show resistance to pests and diseases, and are generally regular croppers producing stable yields. As their flesh is very difficult to separate from the stone, these cultivars are not suitable for either fresh consumption or industrial processing into non-alcoholic beverages. Therefore, they are solely used for brandy making purposes (Nikicevic, 2008).

Plum brandy making technology involves the following operations: harvesting, transport of plums to fermentation vessels, crushing, flesh/stone separation, alcoholic fermentation of the plum mash, distillation of the fermented mash, ageing and finishing.

Apricot cultivars suitable for brandy making include 'Roksana', 'Hungarian Best', 'Ke kemetska Ruža', 'Nagit', 'Cegledi Bibor'.

The apricot brandy making technology involves the following stages: harvesting ripe or overripe fruits, crushing, stone/flesh separation, alcoholic fermentation of fruits in closed vessels, distillation of the fermented mash, ageing and finishing.

Pear cultivars suitable for brandy making include 'Vilijamovka', 'Boskova Bo ica', 'Kleržo', 'Kalu erka', 'Karamanka', 'Krasanka' (Ilic, 1987).

The basic steps in pear brandy making technology include: harvesting, storage for a period of 2-4 weeks, crushing, alcoholic fermentation of fruits, distillation of the fermented mash, ageing and finalisation.

Distillation is the major step in brandy making technology involving the partial or complete separation of a liquid mixture of two or more substances into its component fractions based on their different vapour pressures i.e. boiling points at the same temperature. The main components of a distillate are ethanol, water and a small amount of foreign material (Nikicevic and Teševic, 2008). In the production of strong alcoholic beverages, distillation is not used to separate all volatile components from the initial feed into the distillate or to separate all foreign material from alcohol, but rather to separate alcohol at a favourable alcohol/foreign material ratio, thus conferring the quality and character typical of each type of the initial feed material. Substantial amounts of water and foreign material have an unfavourable effect on distillate quality. Therefore, a crude distillate is generally not used in finished products; rather, it is subjected both to increases in the alcohol content and decreases in the amount of water and foreign matter, through redistillation, deflegmation and rectification.

Ethanol is the key component of distillates, ranking second in dominance after water. Its boiling point is 78.3°C. Ethanol concentration decreases uniformly during distillation.

Acetic acid is the most dominant volatile acid in brandies. The concentration of volatile acids is the lowest in the initial fraction of the distillate, and it increases progressively in the intermediate and final fractions.

Ethyl acetate is the most dominant ester in brandies. It boils at a temperature of 77.2°C. Almost all its content is distilled at the start of the distillation process. Apart from ethyl acetate, brandies contain the following esters: aromatic esters, ethyl caprate, ethyl caproate, ethyl aurate, ethyl lactate.

Organoleptic evaluation includes the following brandy attributes: colour, clarity, typicality, odor and taste.

### Materials and methods

Plum, apricot and pear brandies produced over different years from 1985-2010 in the a ak region were used in the experiment. Analytical quality parameters were assessed by conventional methods: the ethanol content by an alcoholometer; total acids by the volumetric method using 0.1 M NaOH titration; the nonvolatile acids content by the volumetric method after extract determination; volatile acids were calculated from the difference between total acids and nonvolatile acids. Esters were determined by retitration. Organoleptic evaluation was made by students and teachers at the Faculty of Agronomy, a ak, using the positive 20-point scale system.

### Results and discussion

Table 1. Chemical analysis of plum distillates

Year of production	Ethanol % v/v	Total acids g/l	Nonvolatile acids g/l	Volatile acids g/l	Esters mg/laa
1985	55	2.76	0.768	1.992	1520
1997	54	2.52	0.888	1.632	1335
2000	53	2.40	1.032	1.368	1456
2004	54	2.35	0.720	1.632	1494
2007	54	2.28	1.008	1.272	1331
2010	53	2.21	0.528	1.680	892

Table 2. Chemical analysis of apricot distillates

Year of production	Ethanol % v/v	Total acids g/l	Nonvolatile acids g/l	Volatile acids g/l	Esters mg/laa
2004	42	0.360	0.312	0.048	471.4
2007	43	1.368	0.648	0.720	972
2010	43	1.872	1.176	0.696	1309

Table 3. Chemical analysis of pear distillates

Year of production	Ethanol % v/v	Total acids g/l	Nonvolatile acids g/l	Volatile acids g/l	Esters mg/laa
2004	47.5	2.016	0.960	1.056	787
2007	44.5	1.440	0.960	0.480	761
2010	43	0.960	0.672	0.288	956

Table 4. Sensory evaluation of plum, apricot and pear distillates

Sample	Colour 0-1	Clarity 0-2	Typicality 0-2	Odor 0-6	Taste 0-10	
Plum brandy 2010	0.65	1.00	1.55	4.80	7.70	15.70

Plum brandy 2007	0.85	1.00	1.80	5.25	8.85	17.75
Plum brandy 2004	0.90	1.00	1.90	5.60	8.90	18.30
Plum brandy 2000	0.95	1.00	1.95	5.60	8.90	18.40
Plum brandy 1997	0.95	1.00	2.00	5.70	9.20	18.85
Plum brandy 1985	1.00	1.00	2.00	5.80	9.50	19.30
Apricot brandy 2010	0.95	1.00	1.95	5.50	8.60	18.00
Apricot brandy 2007	0.95	1.00	1.95	5.40	8.80	18.10
Apricot brandy 2004	0.95	1.00	2.00	5.80	9.05	18.80
Pear brandy 2010	0.95	1.00	1.85	5.00	8.10	16.90
Pear brandy 2007	0.95	1.00	1.90	5.10	8.25	17.35
Pear brandy 2004	0.95	1.00	1.95	5.25	8.30	17.45

The results of the chemical analysis of the plum distillate samples showed that the 1985 plum brandy had the highest values for the ethanol content (55% v/v), total acids content (2.52 g/l) expressed as acetic acid, volatile acids (1.992 g/l) and ester content (1520 mg/laa), whereas the highest nonvolatile acid content was found in the 2000 plum brandy.

The results of the chemical analysis of apricot distillates showed an identical ethanol content (43% v/v) in the 2007 and 2010 samples, the highest contents of total acids (1.872 g/l), nonvolatile acids (1.176 g/l) and esters (1309 mg/laa) in the 2010 sample and the highest content of volatile acids in the 2007 sample (0.72 g/l).

The results of the chemical analysis of pear distillates showed that the 2004 sample had the highest contents of ethanol (47.5% v/v), total acids (2.016 g/l) and volatile acids (1.056 g/l), whereas the ester content was highest in the 2010 sample (956.5 mg/laa).

The results of the sensory evaluation of plum, apricot and pear distillates revealed the highest sensory performance in the 1985 plum brandy (average score 19.30), 2004 apricot brandy (average score 18.80) and 2004 pear brandy (average score 17.45), respectively.

### Conclusion

The chemical analysis and sensory evaluation of plum, apricot and pear distillates were performed in this study to evaluate the effect of different type of fruit and production year on the quality of the distillates obtained.

The results of the organoleptic assessment of the distillates showed that the average score increased with distillate age, with plum brandy produced in 1985 exhibiting the best sensory attributes.

The results of the chemical analysis of plum, apricot and pear distillates are in agreement with the Rulebook on the Quality of Strong Alcoholic Beverages (Rulebook on Strong Alcoholic Beverages, 2004).

The chemical analysis and sensory evaluation of home-made plum, apricot and pear distillates in the Raška region were conducted to identify potential improvement in brandy making technology and to obtain products that can satisfy the quality requirements of consumers at the local and foreign market levels.

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**ALLELOPATHIC EFFECT OF *XANTHIUM STRUMARIUM* L. AND *ABUTHILON THEOPHRASTI* MED. EXTRACTS ON GERMINATION OF MAIZE AND SOYBEAN SEED**

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**Abstract**

During 2012 allelopathic effects of *Xanthium strumarium* L. and *Abutilon theophrasti* Med. extracts to germination and initial development of maize (*Zea mays* L.), and soybean (*Glycine max* L.) were studied in laboratory conditions. In addition to the Water extracts out of dry mass of the tested weed species, extracts made by use of hexane, ethyl acetate and methanol in different concentrations were also used. The applied concentrations were 10, 20, 30 and 40 g/l of dry matter made out of weed species in the 3-4 leaf stage of development. Inhibiting effect of water extract from dry matter of *Xanthium strumarium* (L.) and methanol extract from which methanol part was evaporated to maize seed epicotyls and hypocotyls length was established. In comparison to the control, the maximum concentration of 40 g / l of the extract made from Water solution of *Abutilon theophrasti* Med. showed inhibitory effect on soybean seed epicotyls and hypocotyls length. The study was conducted in a randomized block design with 4 replications during which 25 seeds of maize and soybean were laid into Petri dishes. The applied extracts made out of dry matter of the both of the studied weed species *Xanthium strumarium* (L.) and *Abutilon theophrasti* Med. reduced maize seed germination for 14.8-26.83% and soybean seed germination for 18.5-35.82%, in comparison to the control in which it was 95% and 92%, respectively. After germination in a climate chamber, epicotyls' and hypocotyls' length of maize and soybean seeds was measured three, six and ten days following spraying by extracts.

**Key words:** allelopathy, extraction, maize, soybean, seed.

**Introduction**

Weeds effect harmfully to crops by releasing phytotoxines from seeds, by decomposition of remainings, leaching and exudates (Narwal, 2004). Plants release harmful chemicals into the environment, reducing growth and establishment of other plants near them: the process known as allelopathy. Allelopathy is natural ecological phenomenon of relationship between organisms which can be applied for control of weeds, pests and diseases in field crops (Ashrafi *et al.*, 2007). Chemical substances with allelopathic properties have also other ecollogical roles, such as defend of plants and regulation of soil biotype that has impact on soil degradation and fertility. These ecosystems, proportionally to the roles of allelopathic chemicals may be increased, reduced or their common functions may be altered (Inderjit *et al.*, 2011). High allelopathic potential of *Abutilon theophrasti* Med. is shown due to inhibition of germination and growth of competitive plants, by which it reaches superior position. Although allelopathic interactions of *Abutilon theophrasti* Med. with other crops have been known for several decades, weak attention is paid to the biochemical interactions of this weed species, Gressel and Holm (1964), that indicates negative allelopathic effect of *Abutilon theophrasti* Med. to soybean, maize and tomato crops (Quasem and Foy C.L., 2001). Shajie and Saffari (2007) established that extract made of leaves and petioles of *Xanthium*

*strumarium* L. significantly reduced germination and growth of maize (*Zea mays* L.), oil rape (*Brassica napus* L.), sesame (*Sesamum indicum* L.), lentil (*Lens culinaris* Medic.) and chickpea (*Cicer arietinum* L.) seedlings. Some researchers studied the effects of Water solutions made from different parts of plants, as well as their impact on other plant species (Kazinczi *et al.*, 2004; Paul and Sultana, 2004; Uremis *et al.*, 2005; Javaid *et al.*, 2006; Xingsiang *et al.*, 2009 and Qian *et al.*, 2010; Konstantinovic *et al.*, 2013). Different allelopathic activity of different parts of the same weed species also differs in its capabilities of harmful effects to germination and beginning growth of cultivated plants (Aziz *et al.*, 2008; Konstantinovic *et al.* 2012). Plant can show inhibiting, but also stimulating effect to germination and growth of the nearby plants. In addition to examples of biochemical competition between weed and crop plants, there are examples of allelopathic interference between cultivated plants. This is the best illustrated by long time applied rotation system *Zea mays* (maize), *Glycine max* (soybean). It is observed that the rotation of these crops provides up to 20% higher yields (Rizvi and Rizvi, 1992).

Allelochemicals can have effect to the change of weed flora composition, crop growth and yield, and potentially can be used as a weed control measure (Singh *et al.*, 2001). Allelochemicals escape from plants in different ways; four major methods by which allelochemicals releases from mother plants could be summarized as: 1) leaching- in this way inhibitor components could be produced by dead or alive parts of plants; 2) volatilization- by which terpenes components are released from the leaves of some plant species; 3) decomposition- in this method allelochemicals are released from plant residue; and 4) exudation-in this way high quantities of organic compounds release from roots of several crop and non-crop species which acts as an inhibitor for the growth of other plants (Gill *et al.*, 1993). Allelopathy may be an important mechanism in the plant invasion process. The lack of co-evolved tolerance and resistance of resident vegetation to new allelochemicals produced by invading weed species could have negative effect to dominant species of natural plant communities (Hierro, 2003).

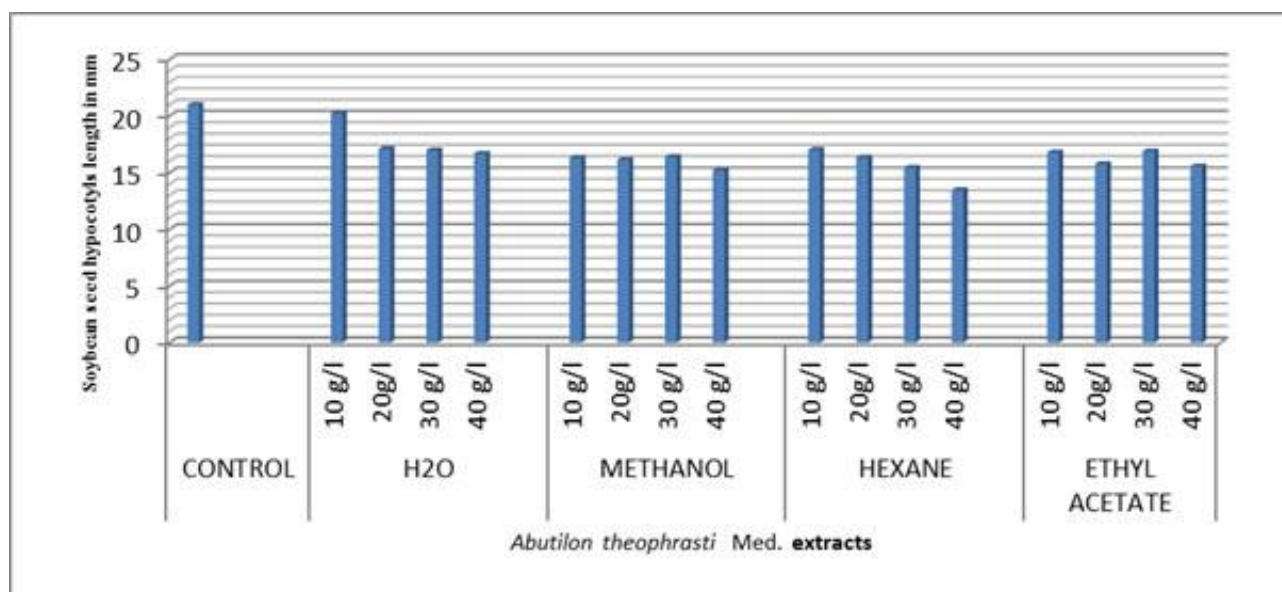
### Material and methods

In 2012, at locality KaC near Novi Sad plant parts of *Abutilon theophrasti* Med. and *Xanthium strumarium* L. (stem and leaf) were collected. Water extract was prepared in the following manner: in 0.5 l of water, 150 g of chopped green mass of leaves and stems of *Abutilon theophrasti* Med. and *Xanthium strumarium* L were immersed. Plant material was left in water for 96 hours at room temperature, after which it was removed, and the extract was filtered by vacuum filter. Filter paper in Petri dishes (150 mm x 25 mm) with germinated seed of the assayed soybean and maize crops was saturated by 8 ml of the extract. Control was moistened by distilled water. The assay was performed according to the method of Šćepanovic *et al.*, 2007. In addition to the applied aqueous extracts, extracts made by use of methanol, hexane and ethyl acetate, from which by evaporation methanol, hexane and ethyl acetate were thrown, were also applied. All four extracts made of the above ground parts of *Abutilon theophrasti* Med. and *Xanthium strumarium* L. were made in concentrations of 10, 20, 30 and 40 g/l. By extracts treated soybean and maize seed were germinated in climatic chamber set to the following parameters: 24 °C for 14 h with illumination (400 Imol) and 22°C for 10h without illumination (Chon *et al.*, 2003). Seed surface was sterilized according to Elemaru and Filhou (2005).

The assays were set up according to the randomized block design with 4 replications. Each Petri dish contained 25 soybean and maize seeds, i.e. 100 seeds per treatment. All measurements were conducted third, sixth and tenth day after moistening of the studied crops seed. The existence of allelopathic activity of these two studied weed species to the soybean and maize crops were established by measurement of the crops seed epicotyls (mm) and hypocotyls (mm) length and germination (%) (Šćepanovic *et al.*, 2007).

## Results and discussion

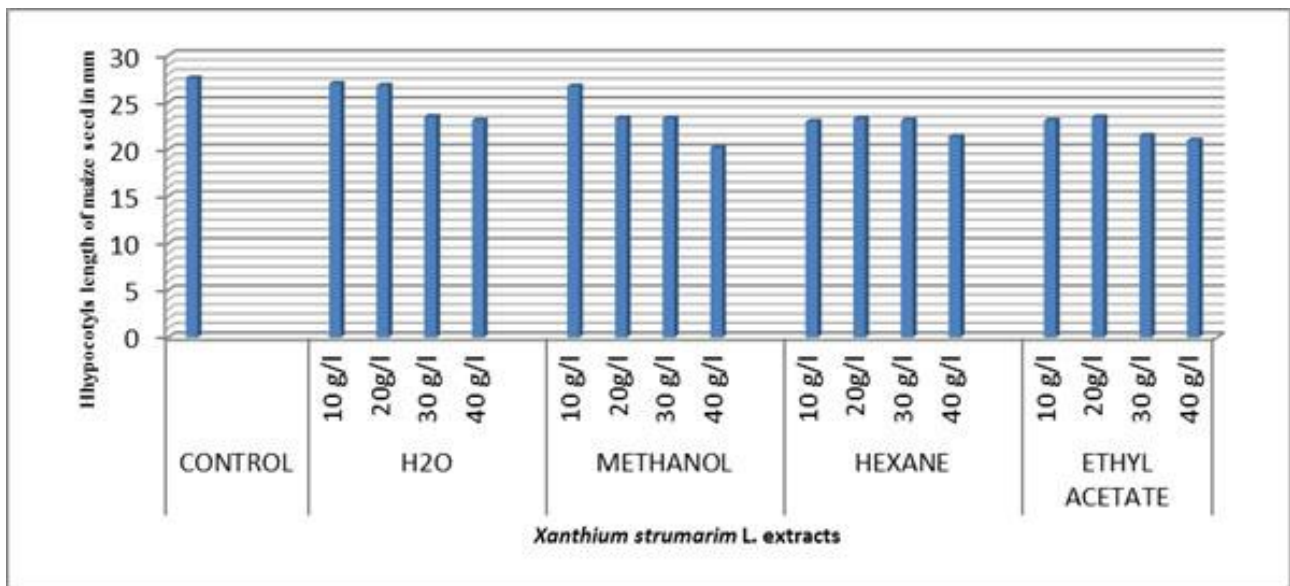
Experimental data confirm results of other authors (Turk and Tawaha, 2002. and Ashrafi *et al.*, 2007), in which allelopathic effects were reflected in inhibition of germination, that was even more pronounced for the growth of seedlings. Germination of the studied maize seed was 95% and soybean seed 92%. (Statistica 10) The significant difference of average values of hypocotyls length was tested by statistical data analysis after treatment with extracts made of the above ground parts of *Abutilon theophrasti* Med. and *Xanthium strumarium* L. In the study, allelopathic effect of weed species *Abutilon theophrasti* Med. and *Xanthium strumarium* L. to the beginning growth stages and development of soybean and maize hypocotyls was confirmed, while epicotyls growth was not statistically significantly different from control values for soybean. Water extracts of the above ground (leaves and stem) parts of *Abutilon theophrasti* Med. in concentrations of 20, 30 and 40 g/l showed inhibiting effects to the length of soybean hypocotyls in values (3.88mm; 4.01mm; 4.3mm), lower in comparison to the control value of 20.98mm. All concentrations of methanol extract, hexane and ethyl acetate showed statistically significant difference in comparison to the control. The highest difference in comparison to the control showed hexane extracts made of *Abutilon theophrasti* Med. in concentration of 40 g/l, with measured hypocotyls length of 13.46mm, and control value of 20.98mm (Graph. 1).



**Figure 1** Measured values of soybean seed hypocotyls after treatment with *Abutilon theophrasti* Med. extracts.

All concentrations of extractions of hexane and ethyl acetate made of *Xanthium strumarium* L. had inhibiting effect of the growth of maize hypocotyls. Hexane extraction of 40 g/l reduced hypocotyls growth for 22.57% in comparison to the control value, while ethyl acetate extract in concentration of 40g/l reduced maize seed hypocotyls for 24% in relation to the control. Water extracts of *Xanthium strumarium* L. had statistically significant effect to the hypocotyls growth in higher concentrations of 30 and 40 g/l, reducing hypocotyls for 14.8% and 16.29%, respectively. Methanol extraction of *Xanthium strumarium* L. did not show statistically significant effect in concentration of 10g/l, but in remaining three concentrations it inhibited maize seed hypocotyls length. Methanol extraction of *Xanthium strumarium* L. in concentration 40g/l showed the best effect, reducing maize seed hypocotyls growth for 26.83% in comparison to the control (Graph 2).





**Figure 2** Measured values of maize seed hypocotyls, after treatment with *Xanthium strumarium* L. extracts.

The increase of maize seed hypocotyls statistically differed in comparison to the control value only in extracts with hexane and methanol in the highest concentrations of 40g/l. Epicotyls length of maize seed treated by hexane extract made of *Xanthium strumarium* L. was 6.17mm and control value was 8.43mm. Epicotyls length of maize seed treated by methanol extract made of *Xanthium strumarium* L. was 5.83mm, and control value was 8.43mm. All other studied extract concentrations did not show statistically significant effect to the epicotyls growth. According to the study of Benyas *et al.*, 2010, only higher concentrations of *Xanthium strumarium* L. water extracts had also effect to the studied lentil crop. According to the studies of Kalinova *et al.*, 2012, water extracts of *Sorghum halepense* (L.) Pers. root had an effect to the germination of soybean, peas and vetch for 28.8% to 86.3%.

### Conclusions

Based upon conducted studies, data were obtained on the effect of effect of *Abutilon theophrasti* Med. and *Xanthium strumarium* L. extracts on hypocotyls and epicotyls length of soybean and maize seed. Effect of *Abutilon theophrasti* Med. extract in tested concentrations of 10, 20, 30 and 40 g/l to the growth of soybean seed hypocotyls was between 18.5% in water extract, up to 35.82% in hexane extract, which was lower in comparison to the control. Effect of *Xanthium strumarium* L. extract in tested concentrations of 10, 20, 30 and 40 g/l to the growth of maize seed hypocotyls was between 14.8% in water extract, up to 26.83% in methanol extract, which was lower in comparison to the control. The tested concentrations did not show significant deviations in epicotyls length except for the two *Xanthium strumarium* L. extracts in the highest concentration of 40g/l.

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## POSSIBILITY OF CHEMICAL WEED CONTROL IN SPRING RAPESEED

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### Abstract

Possibility to chemically control, annual and perennial broad-leaf and narrow-leaf, weeds in spring rapeseed has been tested in the experimental station of Rimski Šan evi (locality: Novi Sad). The following herbicides (a.i.) were tested: trifluralin, clomazone, quizalofop-p-ethyl and clopyralid. Simultaneously we tested the effect of the herbicides on hectoliter weight of seed, as well as oil and protein content in seed. In Novi Sad, the location predominated by annual broadleaf weeds, the performance of these herbicides was much better. The tested herbicides differed significantly in their effect on the quantity and quality on yield parameters of spring rapeseed. Of all the tested herbicides only the application of Gamit (clomazone) showed phytotoxicity that was expressed on plants.

**Key words:** spring rapeseed, weeds, herbicides.

### Introduction

Rapeseed (*Brassica napus* L.) belongs to family *Brassicaceae* and is an important oil-seed crop throughout world (Miri, 2007). In Europe, excluding Sweden, the acreage under spring rapeseed is small (Kondic et al., 2008). Winter rapeseed cultivars are dominant in European countries (Marinkovic et al., 2010), but selection and breeding of spring cultivars intensified in Europe in the second half of the 20<sup>th</sup> century (Mustapic et al., 1984). Canola seeds are not only a rich source of oil (40-45%), but also a source of good quality protein (25%) (Roshdy et al., 2008). Weeds are considered as one of the most important determinative factors in canola fields. They compete with crop plants for light, water, nutrients, space and may have some allelopathis effect.

The spring rapeseed is less competitive against weeds than the winter rapeseed, especially at the beginning of the growing season. The optimum time for spring rapeseed planting is from mid-March to mid-April, a period that coincides with the emergence of germination and spring weeds (Konstadinovic et al., 2007). If seedbed preparation is performed well and the spring rapeseed is planted at optimum date, the crop usually stays weed-free in the early stages of development. However, post-sowing emergence of weed species (15 to 20 days after sowing) can cause reductions in oil yield and quality (Klaa en, 2006).

Chemical weed control is not a mandatory practice in winter rapeseed and it is performed as needed and not on the entire acreage. In our agricultural practice, the rapeseed is considered a competitive crop and herbicide use is not considered cost-effective (Mitrovic et al., 2009). However, questionnaires have shown that herbicides application is practiced both, in the fall and spring (Marisavljevic et al., 2007). The same author recommends that, in addition to chemical treatment before or after sowing and before crop emergence, an additional treatment with metazachlor should be performed after crop emergence, to eradicate the weed that emerged in the mean time. Davies (2005) recommends a similar weed control schedule for the spring rapeseed, the difference being the pre-sowing application of trifluralin instead of clomazone and the post-emergence application of metazachlor and clopyralid.

The objective of this study was to investigate the possibility of controlling weeds in rapeseed plots and to assess the impact of herbicides on quality parameters of spring rapeseed.

### Material and Methods

This study was carried out in 2009 in the experimental station of Rimski Šan evi (locality: Novi Sad). Using the standard method for testing the efficiency of herbicides in rapeseed crops (Anon, 2004). Material for the experiment was spring rapeseed cv. *Jovana*, in property of Institute of Field and Vegetable Crops from Novi Sad, Serbia, registered for commercial use. The experiment was set up as a randomized block design with three replicates. Plot size was 30 m<sup>2</sup>. Basic data for the experiment are shown in tables 1 and 2. Chemical treatment was performed by means of a backpack sprayer "Solo", with an extension tube fitted with eight Lurmark 03 F 110 nozzles. Herbicides were mixed with water, which was applied at a rate of 300 l/ha when rapeseed plants were 10 cm tall and weeds in stage of 2-6 pairs of leaves. In addition to the tested herbicides, the experiment included also two controls – one with and another without hoeing. Control 1 served for the evaluation of effectiveness of the tested herbicides, the other to determine whether the pesticides had a negative impact on grain yield, seed hectoliter weight, and oil and protein contents in seed. The effectiveness of the herbicides was assessed by counting weed plants per m<sup>2</sup> (weeds/m<sup>2</sup>).

Herbicides phytotoxicity for rapeseed was estimated at the time of herbicide efficiency assessment, visually, on the EWRS scale 1-9: 1 - healthy plants with no symptoms, 2–slight phytotoxic symptoms, 3–medium, but clearly recognizable symptoms, 4–pronounced symptoms whose effect on yield is uncertain, 5–strong symptoms, growth disorder, chlorosis perceivable, etc., when yield reduction is expected to occur, 6, 7, 8, 9 –severe damage to complete destruction of plants (Anon, 1981). Foliar application of Lontrel and Globus was made on 20 May 2009, when rapeseed plants were about 10 cm tall and most of the weeds were at the stage of 2–6 developed leaves (at the time of treatment, weed infestation rate was not assessed). Basic statistical calculations of rapeseed quality were done by the *t*-test (Mead et al., 1996). First assessments of weed infestation rate in the crop were done two months after planting, at both sites (Tab. 1).

Table 1. Basic data for the experiment

Location	Novi Sad
Soil type	Degraded chernozem
Previous crop	Seed pea
Planting date	25 Mar 2009
Application date	23 Mar 2009
	Trefgal and Gamit
	18 May 2009
	Lontrel and Globus
Assessment dates	1 <sup>st</sup> assessment: 26 May 2009
Harvest	04 Aug 2009

For foliar treatment, assessments were done 30 days after planting. The reason for a rather late performance of the assessments were poor weather conditions (a spell of extreme drought). In Novi Sad, a total rainfall from the beginning of April till mid-May was 15 l/m<sup>2</sup>, with temperatures soaring up to 30°C in the first half of May. The experiment in Novi Sad was sprinkler irrigated on 10 April to provoke the emergence of rapeseed plants and weeds.

### Results and Discussion

Tables 2, show the results of the first assessments of weed infestation (number of weeds/m<sup>2</sup>) performed in the locations of Novi Sad.

Table 2. Weed infestation rate (No. of weed plants/m<sup>2</sup>) in rapeseed crop, Novi Sad location, [1<sup>st</sup> assessment on 26 May 2009]

Weeds	Treatments							
	1	2	3	4	5	6	7	8
<i>Ambrosia arthemisiifolia</i>	-			1				1
<i>Bromus molis</i>	1							-
<i>Capsela bursa pastoris</i>	-							-
<i>Capsela bursa pastoris</i>	-	-		-				0.5
<i>Cirsium arvense</i>	1							-
<i>Convolvulus arvensis</i>	1.5	1	1		1	3.5		-
<i>Conyza canadiensis</i>	-	1	1*		1	1	1	-
<i>Euphorbia helioscopia</i>	-				1			-
<i>Fumaria officinalia</i>	-			1				1
<i>Lactuca seriola</i>	1	1	1		0.5	3	1	-
<i>Sinapsis arvensis</i>	-			0.5				2
<i>Solanum nigrum</i>	2	1	1	1	2	2.5		1
<i>Sonchus arvensis</i>	1	0.5						-
<i>Stachis annua</i>	-				1			1
<i>Xanthium strumarium</i>			1					
Phytotoxicity		2	2	2-3	2-3	3-4	2	2

\* plants with arrested growth but not destroyed

Table 3. Statistical significance of differences in rapeseed quality parameters between the treated variants and the untreated control variants

Locality: Rimski Šan evi (Novi Sad)		Treatments						
		2	3	4	5	6	7	8
Hectoliter weight (g)	KBK	ns	ns	ns	ns	ns	ns	ns
	KSK	ns	ns	ns	ns	ns	ns	0.039*
Oil content (%)	KBK	ns	ns	ns	ns	ns	ns	ns
	KSK	ns	ns	ns	ns	ns	ns	0.032*
Protein content (%)	KBK	ns	ns	ns	ns	ns	ns	ns
	KSK	ns	ns	ns	ns	ns	ns	ns

P < 0.01\*\*; P < 0.05\*; NS-not significant

KBK–control; KSK–control with manuell hoeing

The herbicide Gamit, in the combination Gamit + Trefgal, exhibited phytotoxicity to the rapeseed crops in locality of R. Šan evi (Fig. 1).



Figure 1. Phytotoxic effect of herbicide Gamit (clomazone) on oilseed rape

The rapeseed plants treated with the combination Trefgal and Gamit in the amounts of 1.5 l/ha + 0.2 l/ha exhibited low phytotoxicity which was manifested as etiolation of individual leaves totalling about 10% of the plant foliage at the stage of 1-3 true leaves. The plants treated with Gamit alone, in the amount of 0.2 l/ha, exhibited similar symptoms. Gamit applied in the quantity 0.3 l/ha caused some what more pronounced symptoms, etiolating about 18–22% of the plants at the stage of 1–3 true leaves. These symptoms are known to occur in response to the application of clomazone-based herbicides, and they are temporary and disappear in the course of further plant growth. The phenomenon was discussed by Davies (2005).

In the experimental plots of Rimski Šan evi (Novi Sad), 15 weed species were registered, one grassy (perennial) and 14 broadleaf weeds (2 perennials and 12 annuals). In this experiment, the combinations Trefgal + Gamit (1.5 l/ha + 0.2 l/ha) and Trefgal + Lontrel (1.5 l/ha + 1.0 l/ha) were most effective in weed control. In this experiment too, certain weeds were unevenly distributed (in patches or as individual plants). The effective performance of pre-emergence application timings of herbicide was observed on reduced growth and population of weeds from the very beginning, which increased seed yield in rapeseed significantly. Similar result has been reported by Khan & Mumtaz (1995), Yadav et al. (1999). and Singh et al. (2001). Application of herbicides decreased the weed density over control. Effectiveness of herbicides in controlling weeds has been reported by Yadav et al. (1999). Bagherani & Shimi (2002) have also reported that among five herbicides (trifluralin, ethalfluralin, cyanazine, alachlor and propyzamide), the most efficient treatment was trifluralin.

### Conclusions

The tested herbicides showed higher efficiency in the location of Novi Sad. Best effects were demonstrated by the combinations Trefgal + Gamit (1.5 l/ha + 0.2 l/ha) and Trefgal + Lontrel (1.5 l/ha + 1.0 l/ha). Trefgal (2.5 l/ha) and Globus (2 l/ha) exhibited a negative effect on protein content in the location of Novi Sad.

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**FIRST REPORT OF *RHIZOCTONIA ZEA* CAUSING STUNTING AND ROOT ROT ON WHEAT IN TURKEY**

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**Abstract**

*Rhizoctonia* is a destructive soilborne pathogen with a wide host range in the world. It is one of the main causal agents of dryland root rot on wheat in Turkey. Wheat is widely planted in the Central Anatolia Region in Turkey. In order to identify species of *Rhizoctonia*, surveys of wheat fields in the Konya, Ankara, Eskisehir, Yozgat and Kırıkkale (provinces in the Central Anatolia Regions) were undertaken. Three of the *Rhizoctonia* isolates collected from necrotic lesions on the root and crown were identified as *Rhizoctonia zea* (teleomorph: *Waitea circinata* var. *zea*), as well four of the isolates from rhizosphere soils. Species identification were done according to the basis of hyphal and colony morphology, anastomosis reaction with known tester isolates and comparing sequences of Internal Transcribed Spacer (ITS) region. Colonies growth on PDA were orange when young and became salmon colored with age. Sclerotia were uniform and nearly spherical, mostly 0.2 to 0.5 mm in diameter, initially orange and turned brown during time. The number of nucleus in each hypha cell was 4 to 8. The resulting sequences were compared to other sequences and were 82 to 95% identical to other *R. zea* sequences in GenBank.

First pathogenicity test was conducted with agar- plate assay with all isolates and then it was tested on seedlings grown in pots the most virulent isolate on susceptible wheat cultivar. Test was done in the greenhouse conditions at  $23 \pm 2$  °C, with a 12-h photoperiod and 50–60% RH. Average disease severity value was determined as 81%. Pathogenicity tests revealed that *Rhizoctonia zea* caused significant reduction of emergence, stunting, reduction in the number of seminal roots and superficial discolouration on the hypocotyls and roots on wheat. Non-inoculated plants remained healthy.

This is the first report of *R. zea* isolated from wheat plants and rhizosphere soils in Turkey

**Keywords:** *Rhizoctonia zea*, first report wheat, Turkey

**Introduction**

*Rhizoctonia* is a destructive soilborne pathogen with a wide host range in the world. It is one of the main causal agents of dryland root rot on wheat in Turkey (Tunalı *et al.*, 2008). Wheat is widely planted in the Central Anatolia Region that included the Konya, Ankara, Eskisehir, Yozgat and Kırıkkale provinces. *Rhizoctonia zea*, the anamorph of *Waitea circinata* var. *zea*, was initially described as a corn pathogen in USA, India, Argentina and China (Voorhees, 1934; Sumner and Bell 1982; Gunnell, 1986; Li *et al.*, 1998). Later, this fungus was also reported to cause disease on other important crops such as sugarbeet, wheat, bean, soybean and turfgrasses (Kuznia and Windels 1994; Tredway and Burpee 2001; Erper *et al.*, 2005).

*R. zea* is the causal agent of reduced total emergences and stands of wheat (Kuznia and Windels, 1994). Although this fungus was previously reported in Turkey from Johnsongrass (Demirci and Eken, 1999), corn kernels (Demirci and Kordali, 1999), bean and soybean roots (Erper *et al.*, 2005), this is the first report of *R. zea* isolated from wheat plants and rhizosphere soils.

## Materials and Methods

### Plant collection and isolation

In order to determine the anastomosis groups and pathogenicity of *Rhizoctonia* species associated with stunting, bare patch, root and crown rot of wheat in Turkey, 330 samples of wheat and rhizosphere soil were collected in 2009 and 2010 growing seasons. Samples were taken from fields in 58 districts of Konya, Ankara, Yozgat, Eskişehir and Kırıkkale provinces. Segments of necrosed tissue were placed on acidified water agar (1.5 % water agar amended 3 ml of 10 % lactic acid per liter of medium). Hyphal tips of *Rhizoctonia*-like fungi were transferred to Potato Dextrose Agar (PDA, Merck, Germany) containing 50 mg/l streptomycin sulfate. Sterile wheat straws were used for *Rhizoctonia* spp. isolation from soil samples. Soil samples (collected with plants) from the respective fields were transferred to pots on a greenhouse bench ( $20\pm 2^{\circ}\text{C}$ ). Plots were then watered to field capacity. About 4 cm long internodal segments of mature, dried wheat straw were inserted vertically, 4 per pot, and left for 3 or 4 days. After that straws were removed, washed, blotted and placed on acidified water agar. Isolates of *Rhizoctonia* were transferred to PDA (Ogoshi *et al.*, 1990).

### Identification

In order to determine hyphal diameter and the number of nuclei per cell of the isolates, *Rhizoctonia* isolates were maintained on PDA in an incubator regulated at  $25^{\circ}\text{C}$  and in darkness. Developing mycelia were stained with safranin O (Sigma, USA) and 3% KOH (Bandoni, 1979) and observed under phase contrast microscopy at  $\times 400$  magnification. Hyphal diameter was determined by measuring 10 cells. Nuclei were counted in 15 cells.

Anastomosis was tested by pairing isolates with representative testers of *Waitea* spp. *Rhizoctonia* isolates and *Waitea* tester isolates (Rz 590: *R. zaeae*; Ro 231: *R. oryzae*, W 616: *Waitea circinata* var. *circinata*) were activated on PDA at  $25^{\circ}\text{C}$  in the dark. Coverslips, sterilized by dipping in 95 % ethyl alcohol and flaming, were coated with a thin layer of 0.5 % PDA and placed on water agar plates. Agar plugs with mycelia of *Rhizoctonia* isolates and the tester isolates were cut the margin of a growing colony and transferred to water agar plates on the opposite sides of the coverslip. After incubation at  $25^{\circ}\text{C}$  for 24–48 h in the dark, when overlapping mycelia of two isolates were observed, the coverslip was removed from the plate and placed on a slide in the mixture of one drop of safranin O and one drop of 3 % KOH. Stained hyphae were observed microscopically. Anastomosing hyphae were traced back to their source in order to confirm the anastomosis between our isolates and the tester isolates (Kronland and Stanghellini, 1988). For the anastomosis testing, all pairs were examined twice.

### Pathogenicity tests

First pathogenicity test was conducted with agar-plate assay with all isolates. Pathogenicity was also tested on seedlings grown in pots the most virulent isolate on susceptible wheat cultivar (cv. Sultan 95). Seedlings were grown in plastic pots (5 cm in diameter) in the greenhouse conditions at  $23 \pm 2^{\circ}\text{C}$  with a 12-h photoperiod and 50–60 % RH. Inoculum was prepared on the moistened sterile wheat grains in test tubes. The bottom of the pots were filled with  $40\text{ cm}^3$  of medium vermiculite and topped with  $30\text{ cm}^3$  of natural sterile silt loam. Eight pathogen-colonized wheat grains were placed in the middle of the soil layer. Control consisted of pots without inoculum. All pots were covered with clear polyethylene and incubated 5 days. There were six replicate pots for treatment. After 5 days, eight seeds of wheat were placed on the soil surface, covered with  $10\text{ cm}^3$  of steril natural topsoil, and watered with 10 ml of distilled water. After 3 weeks, the plants

were washed free of soil (Paulitz *et al.*, 2003). Severity of *Rhizoctonia* root rot was evaluated on a scale of 0 to 8 (Kim *et al.*, 1997).

### Molecular Analysis

Approximately, 300 mg mycelium were harvested and ground with liquid nitrogen in a sterile mortar for DNA extraction from culture medium. Genomic DNA was extracted using a Qiagen DNeasy<sup>®</sup> Plant Mini Kit, as specified by the manufacturer, and stored at -20 °C prior to use.

PCR reaction mixtures and condition were modified from previous studies (Aroca and Raposo 2007; Cobos and Martin, 2008). The reaction mixtures of PCR, a final volume of 50 µl, contained 5 µl of 10X buffer [75 mM Tris HCl, pH 9.0, 50 mM KCl, 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>], 2 µl of 5 µM each primers, 5 µl of 1.5mM MgCl<sub>2</sub>, 2 µl of 10 mM deoxynucleoside triphosphates (dNTPs), 1 U Taq polymerase (Fermatas), 5 µl of DNA template for each reaction and 5 µl of bovine serum albumin (BSA: 10 mg/ml). DNA amplifications were carried out in a Techne TC-5000 thermal cycler by the following program: 94 °C for 2 min, followed by 34 cycles of (1) denaturation (94 °C for 30 s), (2) annealing (60 °C for 30 s) and (3) extension (72 °C for 30 s), and a final extension step 10 min at 72 °C.

The ITS region of the isolate was amplified using the universal primers ITS-1 (5' TCC GTA GGT GAA CCT GCGG 3') and ITS-4 (5' TCC TCC GCT TAT TGA TATGC 3'). The PCR products were separated in 1.5 % agarose gels stained with ethidium bromide, and visualized under UV light. They were sequenced by REFGEN (Gene Research and Biotechnology Company, Ankara, Turkey).

### Results and Discussion

The nucleus number that was found in each hypha cell was 4-8, and width of the main runner hyphae was more than 7µm. According to the cellular nucleus number, width of the main runner hyphae, colony morphology and the anastomosis test, seven isolates were identified as *R. zeae*. As a result of anastomosis test with testers isolates of *Waitea* spp, these isolates anastomosed with high fusion frequency (C 3 reaction) with tester isolates Rz 590 belonging to *R. zeae* (Fig.1).



Figure 1. 28-day old colony appearance of *Rhizoctonia zeae* on potato dextrose agar (a); anastomosis between hyphae (b)

Morphological features of isolates on PDA were similar with descriptions of Demirci (1998) and Telmadarrehei *et al.*(2011). Colonies were orange when young, turning salmon coloured with age. Sclerotia were uniform and nearly spherical, mostly 0.2 to 0.5 mm in diameter, initially orange and turned brown during time .

The resulting sequences were compared to other *Rhizoctonia* sequences and were 82 to 95% identical to other *R. zea* sequences in the GenBank. They were submitted to the GenBank with the accession numbers of KC590513, KC590514, KC590515, KC590516, KC590517, KC590518 and KC590587.

Regarding the pathogenicity test on agar plates, all seven *R. zea* isolates were found to be pathogenic (72- 98%) on susceptible wheat cultivar. The most virulent isolate tested in plastic pots in greenhouse conditions and average disease severity value, was found to be 81%. *R. zea* isolate caused significant reduction of emergence, stunting, reduction in the number of seminal roots and superficial discolouration on the hypocotyls and roots on wheat. Non-inoculated plants remained healthy. The pathogen was reisolated from diseased plants.

*Rhizoctonia zea* was described for the first time in 1934 in Florida (USA) as causing agent of ear rot of corn (*Zea mays* L.) (Voorhees, 1934). The fungus has also been reported on the other important crops, such as wheat, although there are few publications related to *R. zea* on wheat in the world (Kuznia and Windels 1994; Telmadarrehei *et al.*, 2011). *R. zea* is the causal agent of reduced total emergences, stands and eye spot of wheat in USA and Iran (Kuznia and Windels 1994; Telmadarrehei *et al.*, 2011). Result of pathogenicity test presented in this study, similarly as results of Kuznia and Windels (1994), showed that *Rhizoctonia zea* caused significant reduction of emergence, stunting, reduction in the number of seminal roots and superficial discolouration on the hypocotyls and roots on wheat.

### Conclusion

*Rhizoctonia* is one of the main causal agents of dryland root rot on wheat in Turkey. In present study the *Rhizoctonia* isolates, obtained from wheat, were identified as *R. zea*. It is known that the host range and diseases caused by the isolates from different species or anastomosis group are different. Therefore, determination of the species or anastomosis grouping of the isolates is very important. Results of this study present the first report of *R. zea*, causing disease of wheat in Turkey. Due to the severe symptoms, it should be considered a potential threat to wheat cultivation in Turkey.

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## COLEOPTERAN PESTS INTERCEPTED ON IMPORTED FOREST PRODUCTS IN TURKEY

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### Abstract

Five species of pests were intercepted in timbers imported to Turkey. All of them have plant quarantine importance were determined by Plant Protection Central Research Institute in Ankara. Intercepted pests were identified by an entomologists and taxonomists. These pests was identified as *Scolytus multistriatus* (Marsham, 1802) and *Scolytus ratzeburgi* (Janson, 1856) (Coleoptera: Curculionidae: Scolytinae) were intercepted from Ukrain in 2009. Another pest was identified as *Trichoferus campestris* (Faldermann) (Col.: Cerambycidae). It was intercepted on timber imported from Rusia in 2011. The other pest *Monachamus galliprovincialis* (Olivier) (Col.: Cerambycidae) was intercepted on industrial wood imported from Ukrain in 2011. The last one *Ips acuminatus* Gyllendal (Col.: Scolytidae) was imported from Ukrain in 2012. According to these results, quarantine inspectors at the checkpoints of the plants and plant products entrance gates must be careful during inspections of imported forest products in Turkey. In this study, hosts, damage, geographical distribution, pathways, pest significance and phytosanitary measures were evaluated of these pests.

**Key words:** Coleoptera, Forest product, Interception, Quarantine, Turkey

### Introduction

In Turkey, forests cover 21.189.000 ha area and %27, 2 percent of overall country area. Forests are consist of % 60 percentage of conifers such as; Turkish pine (*Pinus brutia*) and by order of European black pine (*Pinus nigra*), Scots pine (*Pinus silvestris*), Fir (*Abies sp.*), Oriental spruce (*Picea orientalis*) and Cedar (*Cedrus libani*) and % 40 percentage of deciduous tree species such as Oak (*Quercus sp.*), Beech (*Fagus sp.*), Black alder (*Alnus glutinosa*), Chestnut (*Castanea sativa*), Muscledwood (*Carpinus sp.*).

Forest product includes both coniferous and nonconiferous species. *Coniferous species* contain all woods derived from trees classified botanically as Gymnospermae, for example; fir (*Abies*), parana pine (*Araucaria*), deodar (*Cedrus*), ginkgo (*Ginkgo*), larch (*Larix*), spruce (*Picea*), pine (*Pinus*), etc. These are also generally referred to as softwoods. *Non-coniferous species* contain all woods derived from trees classified botanically as Angiospermae, e.g. maple (*Acer*), alder (*Alnus*), ebony (*Diospyros*), beech (*Fagus*), lignum vitae (*Guaiacum*), poplar (*Populus*), oak (*Quercus*). These are generally referred to as broadleaves or hardwoods (<http://www.fao.org>).

In recent years, international trade of forest products has been increased and caused introductions of foreign exotic organisms. Some of the potential organisms may not be considered as pests in their place of origin; however, they may reach pest status in a different environment. It has known that most of the pests are spread out from one country to another by the importation of raw materials of trees. In case of importation of logs, timber and woods the agricultural quarantine regulations are applied to these products which are imported into Turkey. There are also some requirements which are used for the wood packaging materials.

Quarantine pests has potential economic importance to an area which is not yet present in their country, or present but not widely distributed, and are being officially controlled. Official

visual examination of plants and forest products are made by the inspectors. All quarantine pests are regulated organisms, that is, phytosanitary action should be taken if they are intercepted. If the forestry products infested with the harmful organisms that constitute a barrier for importation and are limited and prohibited to importation. There are some Coleopteran species take places in the Regulation on Agricultural Quarantine of Turkey which contains harmful organisms importation is prohibited in case of found in some plants and plant products.

### Materials and methods

This study was conducted during 2009 and 2013 in Ankara, Turkey. Aim was to identify of the wood destroying insects in an imported forest products to Turkey. Insect species such as *Scolytus multistriatus*, *Scolytus ratzeburgi*, *Ips acuminatus*, *Trichoferus campestris* and *Monochamus galliprovicialis* were assessed to as material. The other materials were timbers and industrial woods. Insect samples were taken from infested timbers and industrial woods. Infested wood samples and insect samples were sent from quarantine laboratories to Plant Protection Central Research Institute for identification. Some of the wood samples were inspected at the laboratory and placed in screen cages for insect emergence. Emerged insects were collected and identified by the taxonomists.

### Results and discussion

Many important pest species are known to spread through forest trees from country to country and continents by importation. The probability of pests being with host are detected at the point of entry under present quarantine procedures. For instance, *Scolytus multistriatus*, *Scolytus ratzeburgi*, *Ips acuminatus*, *Trichoferus campestris* and *Monochamus galliprovicialis* were intercepted by inspectors at the border in Turkey. Inspection was made by the inspectors and sample was taken from the imported wood products which were infested with the insects.

Plants and plant products are damaged by insect species feeding on leaves, opening galleries on buds, branches and stems, sucking and feeding on roots. Among the pests in timbers of conifers such as Scolytidae and Cerambycidae species are the most common insect pests. *Scolytus* spp. and *Ips* spp. are very important pests for forests in Scolytidae family. They cause the death of trees and serious economic losses on timber which is also destroyed. Common features of bark beetles; they live phloem under the bark layer or galleries in wood. Larva creates special structural galleries. This galleries structure is significant characters for species and is used for identification of the pest.

The other species *Monochamus* spp. and *Trichoferus campestris* are belonging to the Cerambycidae family. These insects oviposit on recently dead and felled trees or trees already under stress. The feeding of the larvae produces feeding tracks on the sapwood under the bark and bore holes into the wood which may make the wood unsaleable. From the exterior, the conical oviposition scars on the bark can show that a tree has been attacked. With the bark removed, young larvae can be seen producing feeding tracks in the sapwood. The oval entry holes in the wood caused by older larvae are characteristic, although they may be hidden by an accumulation of wood borings. Grub holes are elliptical and adult exit holes are circular and their presence indicates that the insects have completed their development in the wood and have departed. *Monochamus* spp. are also the vectors for *Bursaphelenchus xylophilus*, the Pinewood Nematode. This nematode feeds on fungi inoculated into a weakened tree, but can also kill susceptible healthy pine trees, causing a condition called pine wilt disease (<http://www.eppo.int/quarantine>). In this study, host plants, damage, geographical distribution, pathways, pest significance and phytosanitary measures were evaluated of intercepted pests in Turkey.

#### *I. Scolytus multistriatus* (Marsham, 1802) Coleoptera: Curculionidae: Scolytinae

It is known as the Smaller Elm Bark Beetle.

**Host plants:** *Ulmus* spp. (Elm tree). *Zelkova carpinifolia*, *Populus tremula*, *Alnus* sp., *Carpinus betulus*, *Fagus orientalis*, *Prunus domestica*, *P. avium*, *Rhamnus alaternus*, *Quercus* sp. and *Fraxinus excelsior* (Selmi, 1998).

**Damage:** Beetles is vectoring *Ophiostoma (Ceratocystis) ulmi*, the causal agent of Dutch elm disease. Beetles emerged from diseased elms and fly to healthy elms to feed carrying spores of the fungus. Larvae live in galleries. The foliage of diseased branches wilts, turns yellow, then brown, and finally dies. Exiting beetles leave numerous pin-sized holes in the bark.

The diagnostic key is brown streaks in infected sapwood (<http://www.entomology.umn.edu>).

**Geographical distribution:** Europe; (excl. USSR), Austria, Belgium, Britain, Bulgaria, Corsica, Czechoslovakia, France, Germany, Greece, Hungary, Italy, Netherlands, Poland, Portugal, Romania, Spain, Switzerland, Former Yugoslavia, Turkey (Selmi, 1998).

Asia; (excl. USSR), Iran, Africa; Algeria, Egypt, North America; Canada, U.S.A.

**Pathways:** Likely pathway is seeds, nursery stock, bark, lumber, wood packaging material including dunnage and insects.

**Pest significance:** It is a vector of Dutch elm disease *Ophiostoma ulmi* (Buism.) (<http://www.entomology.umn.edu>).

## 2. *Scolytus ratzeburgi* (Janson, 1856) (Coleoptera: Curculionidae: Scolytinae)

It is known as the Birch Bark Beetle.

**Host plants:** The primary breeding hosts are species of oak, *Quercus*, such as *Q. petraea*, *Q. robur* and *Q. dalechamps*. Other tree species such as *Aesculus hippocastanum*, *Betula verrucosa*, *Carpinus betulinus*, *Castanea sativa*, *Corylus* sp., *Fagus sylvatica*, *Ostrya carpinifolia*, *Populus* spp., *Salix* spp., *Sorbus* spp. and *Ulmus* spp. have been cited as occasional hosts.

**Damage:** Pathogenic fungal diseases as *Ceratocystis ulmi* (Buisman, 1932) transmitted by *Scolytus multistriatus* (Marsham, 1802), the agent of the blue stain wood.

**Geographical distribution:** In the Baltic States and Northern Europe *Scolytus ratzeburgi* can be traced in the whole region. In Belarus as well the species is known from the whole territory of the country (Lazdans 2009).

**Pathways:** Likely pathway is seeds, nursery stock, bark, lumber, wood packaging material.

## 3. *Ips acuminatus* Gyllendal (Coleoptera: Curculionidae: Scolytinae)

It is known as Engraver Beetle.

**Host plants:** Pines, *Pinus* spp. are the predominant hosts of this insect. In Europe and the Near East, Scotch pine, *Pinus sylvestris*, Austrian pine, *Pinus nigra*, Swiss stone pine, *Pinus cembra* and mugo pine, *Pinus mugo* are reported hosts. In China, Korea and Mongolia, *Pinus armandi*, *Pinus koraiensis*, *Pinus sylvestris* var. *mongolica* and *Pinus tabulaeformis* are reported hosts. In Thailand, *Pinus merkusii* and *Pinus caribaea* (exotic) are hosts. Other conifer hosts in Europe and Asia include: *Abies normandiana* (= *A. bormuelleriana*), *Larix decidua*, *Picea obovata* and *Picea orientalis*.

**Damage:** The first indication of attack by bark beetles, including *Ips acuminatus*, is that infested trees fade from green to yellow to reddish brown. Killing of only the upper portion of the crown of pines is a common occurrence in some parts of this insect's natural range.

**Geographical distribution:** Asia: *Ips acuminatus* occurs from Turkey across Russia to China and south to Thailand. This insect is widely distributed in China.-It is also reported from Japan, Korea, Mongolia, Syria, Taiwan, Thailand, Turkey and Russia (Sakhalin Island and Siberia). Europe: Widely distributed in Europe including Austria, Belgium, Bulgaria, Czech Republic, Denmark, England, Estonia, Finland, Germany, Greece, Hungary, Italy, Latvia, Luxembourg,



Netherlands, Norway, Poland, Romania, Scotland, Sweden, Spain, Switzerland, European Russia, Former Yugoslav Republics and the Republic of Georgia.

**Pathways:** Adult beetles are strong fliers capable of flying several km in search of suitable host material. They are also subject to dispersal by winds.

Immature stages (larvae and pupae) and adults can be transported in unprocessed logs, wood products or wooden packing material, dunnage or pallets containing bark strips.

**Pest significance:** *Ips acuminatus* is generally regarded as a secondary insect that attacks weakened or windthrown trees. When populations build up in weakened or down material, they can attack relatively healthy trees. In some instances, *Ips acuminatus* can kill large numbers of trees and cause a significant loss of commercial pine volume.

#### 4. *Trichopherus campestris* (Faldermann) (Coleoptera: Cerambycidae)

It is known as Chinese Longhorn Beetle. It is synonym with *Hesperophanes campestris*.

*T. campestris* was intercepted by inspectors at the border for the first time in Turkey. It was found in a timber which is imported. It is not known to occur in Turkey and it does not exist at the plant quarantine list.

**Hosts:** This species can potentially attack most woody plants. About 40 genera of woody plants, both conifers and angiosperms, are known to be larval hosts for *T. campestris*. (Clarke, 2004). *Hesperophanes campestris* preferentially attacks *Malus* (apple) and *Morus* (mulberry), but has also been recorded on *Betula*, *Broussonetia*, *Gleditsia*, *Salix*, *Sorbus* and various other fruit and deciduous trees. In Japan, it is said to be highly polyphagous. According to some observations, it may attack cut wood of *Picea* and *Pinus* and even structural timbers in buildings.

**Damage:** The characteristic symptoms of infestation by *H. campestris* are: large entrance and emergence holes in trunks, peeling bark, waste from borings at the base of infested trees, tunnels made by large larvae. The leaves of attacked trees often show yellowing and wilting.

**Geographical distribution:** EPPO region: Armenia, Kazakhstan (southern), Kyrgyzstan, Russia (south-east of European part, Transbaikalia, Eastern Siberia, Far East), Uzbekistan.

Asia: Armenia, China (northern), Japan, Kazakhstan (southern), Korea (People's Republic), Kyrgyzstan, Mongolia, Russia (Transbaikalia, Eastern Siberia, Far East), Tajikistan, Uzbekistan. EU: Absent. (Eppo Bulletin 2009). This longhorned beetle is native to Asia. Various life stages of specimens in the *Trichopherus* genus were the fourth most commonly intercepted longhorned beetles in solid wood packaging material transported to North America in 1985 and 2000. Adults were detected in Quebec Canada in 2002 and 2006. (<http://extension.entm.purdue.edu/publications/WB-2.pdf>).

**Pathways:** Natural spread of *H. campestris*, by flying adults, is rapid. Because larvae of *H. campestris* may be hidden in wood and therefore difficult to detect, they may easily be transported with bonsai plants, or wood products, of host plants moving in trade. They may in particular be carried in wood packaging because of their ability to colonize dry wood. Adults could also possibly be carried as contaminants of plants for planting (Eppo Bulletin 2009).

The larvae of *T. campestris* are found under the bark and in dry dead wood and complete their development in two or more years. Wood and solid wood-packaging materials are important as a pathway of introduction for wood-boring species. This species is often intercepted in North America from wood-packaging material of Asian origin, as well as its previous introductions into Europe and North America, it is plausible to assume that *T. campestris* has a high likelihood of becoming established in the temperate regions of the NewWorld (Grebennikov et. al. 2010).

**Pest significance:** *H. campestris* is an A1 quarantine pest for Canada, and also appears in the regulated pest list of New Zealand (with other *Hesperophanes* spp.). In the area of its present distribution, it is considered as a serious pest of forests and orchards, and especially of dry wood. It is most likely to establish in the southern countries of the EPPO region where climatic conditions

favour large cerambycids and a wide range of host plants is available. The main risk of entry is with wood packaging, since the pest is able to develop in dry wood. *H. campestris* is rather unlikely to be transported in small plants for planting (with the possible exception of bonsai plants), since it does not attack small branches, trunks or rootstocks. Large plants of its hosts are rarely traded (Eppo Bulletin 2009). The species is considered a quarantine pest in Europe and is included by the European and Mediterranean Plant Protection Organization (EPPO) in the EPPO A2 List of pests recommended for regulation (Anonymous 2007, 2008).

**Phytosanitary measures:** *T. campestris* is able to attack healthy or slightly stressed trees of many important species. It prefers to attack mature trees and, even if it does not kill them, the infestation results in significant loss of vigor, of wood marketability (because of the boreholes) and of fruit yield in the case of orchards. The pest also has the potential to damage amenity trees in cities. Nevertheless, the relative importance of *T. campestris* in damaging forest trees, trees in natural environments, orchard trees and amenity trees does not appear to have been evaluated in any detail, beyond the observation that the preferred hosts are fruit or amenity trees (*Malus* and *Morus*). *T. campestris* is able to develop in very dry wood, is an important technical pest of wood in the area of its present distribution. Synonym of *T. campestris* which is *H. campestris* was added in 2007 to the EPPO A2 list and endangered EPPO countries are therefore recommended to regulate it as a quarantine pest. Wood packaging should respect ISPM no. 15 (ICPM, 2003). International movement of wood of the host plants seems relatively unlikely, but measures in that case could be debarking, plus grubhole freedom, or kiln drying, or other treatment (Eppo Bulletin 2009).

##### 5. *Monochamus galliprovialis* (Olivier) (Coleoptera: Cerambycidae)

It is known as Pine Sawyer Beetles. It is in the EU Annex designation: I/A1 - as *Monochamus* spp. (non-European)

**Host plants:** Species of *Pinus*, *Picea*, *Abies* and Douglas-fir. The *Monochamus* spp. which are known as vectors of *B. xylophilus* mainly develop in *Pinus* spp. but other coniferous genera can sometimes also act as hosts (<http://www.eppo.int/quarantine>).

**Damage:** *Monochamus* spp. only oviposits on recently felled trees or trees already under stress. The feeding of the larvae produces feeding tracks on the sapwood under the bark and bore holes into the wood which may make the wood unsaleable. The pine wood nematode (*Bursaphelenchus xylophilus*) (Aphelenchoididae) living in coniferous wood of different tree species, a destructive pest of pine trees is very important.

Larval borings can damage freshly cut logs and reduce value of lumber. Larvae bore in wood of dead and dying trees, can cause degrade of lumber. Adults feed on needles and bark of young twigs. Larvae are often found in pine, spruce or Douglas-fir firewood (<http://www.colostate.edu>).

**Geographical distribution:** Various species are found throughout the conifer forests of the northern hemisphere (North America, Europe and Asia).

**Pest significance:** Inspection of timber does not always reveal the presence of insect larvae or pupae, which can be hidden within internal galleries. *Monochamus* larvae can cause economic losses to felled logs by forming bore holes in the wood. This is normally only of significance if the logs are left for a long time in the forest after felling. Under well-managed forest conditions, *Monochamus* spp. is not generally considered to be serious pests in their own right. Thus, the only economic impact arises in countries where *Bursaphelenchus xylophilus* is present and damaging and the *Monochamus* species concerned transmit it (<http://www.eppo.int/quarantine>).

**Phytosanitary measures:**

EPPO's recommendations to prevent the introduction of *B. xylophilus* and its vectors cover plants and wood of all conifers, apart from *Thuja plicata*, from countries where the nematode occurs. It is recommended that coniferous plants should be prohibited but that countries may choose whether to prohibit wood. If not prohibited, wood must have been heat treated to a core temperature of 56°C

for 30 minutes. In the case of packing wood (crates, dunnage etc.), kiln drying could be accepted instead, whereas for particle wood, the alternative of fumigation is also acceptable.

Several quarantine treatments for wood chips have been proposed, such as steam/heat treatment or fumigation in transit with phosphine. The only known effective treatment for wood already infected with *B. xylophilus* and its vectors appears to be heat treatment, in which all parts of the wood reach a temperature of 56°C for at least 30 min; commercial kiln practices normally achieve this (<http://www.eppo.int/quarantine>).

### Conclusion

Forest products are imported in the form of such as timber, log, chips, roundwood and sawnwood. In Turkey during quarantine inspections five species of Coleopteran pests were intercepted in timbers and industrial wood during period between 2009 to 2012 years. All of the harmful insects found in the imported forest products were identified as bark beetles (Coleoptera: Curculionidae: Scolytinae) and longhorned beetles (Coleoptera: Cerambycidae). They are known as the most common wood-destroying insects (Canakcioglu and Mol, 1998; Toper, 2007). Intercepted pests were identified as *Scolytus multistriatus* (Marsham, 1802) and *Scolytus ratzeburgi* (Janson, 1856) *Ips acuminatus* Gyllendal (Coleoptera: Curculionidae: Scolytinae), *Trichoferus campestris* (Faldermann) and *Monachamus galliprovialis* (Olivier) (Col.: Cerambycidae) by an insect taxonomist with the expertise of Coleoptera.

Yalinkılıç and Serez (1992) suggested that exotic pests may be introduced very easily in forest products imported into Turkey because there is a big gap between wood production and demand in Turkey. To close this gap, Turkey has to import wood products from wood exporting countries. The importation of wood from different countries, increases the possibility of the inadvertent introduction of exotic pests into Turkey. To eliminate this possibility, precautionary measures should be evaluated carefully to prevent the inadvertent introduction of exotic pests, and phytosanitary inspection at busy entry points should be performed more carefully. Plants and plant materials are inspected by the plant health inspectorate in order to satisfy plant health requirements. This inspection is conducted during the importation for imported forest products.

In conclusion, importing unprocessed logs from infested countries can have serious damages because of the introduction of forest pests. Measures must be implemented to mitigate the risk of pest introduction and establishment. Pre-border measures are intended to reduce, border measures are intended to intercept and Post-entry measures are a range of regulatory tools that can be used to prevent invasive alien species (Burgiel 2006).

Great attention is needed to make an inspection in order to prevent or reduce introduction of the pests and infestation of forests. To prevent the introduction of harmful organisms occurring from the trade of wood products sufficient phytosanitary measures should be taken at the place of origin.

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## OCCURRENCE OF *VIBRIO SPP.* IN BIVALVE MOLLUSCS HARVESTED FROM BUTRINTI LAGOON, ALBANIA

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### Abstract

The main production of molluscs in Albania consists in the cultivation of *Mytilus galloprovincialis*, which takes place in Butrinti lagoon. All the positive cases of *Vibrio* spp. were isolated between June-September, which is related to the fact that *Vibrio* species prefer high water temperatures. By analyzing the physical and chemical indicators of water it was observed that the above mentioned species of *Vibrio* were isolated in the average temperature of 26.2 °C, average pH of 8.42 and average salinity 28.97 ‰. Based on the results obtained from this study, *Vibrio* species isolated from Butrinti lagoon molluscs were adapted to the following physical-chemical water conditions: temperature intervals of 23.6-28 °C, pH of 8.15 to 8.8 and salinity of 22.1-33.7 ‰.

**Key words:** Bivalve molluscs, Butrinti lagoon, *Vibrio* spp.

### Introduction

Fish and mussels result to be the second source of proteins for consumers behind meat products. However, all over the world have raised concerns about health risks derived from mussels contaminated with various pathogens (2). Although most reports of outbreaks of food infections came from the United States, there are several reports from Europe, Australia and Asia. Since the end of 1800 there has been over 400 epidemic diseases from food origin and over 14 000 cases of gastroenteritis associated with the consumption of contaminated shellfish (3).

Molluscs in Albania occupy a significant place among fishery products. The main production consists in the cultivation of *Mytilus galloprovincialis*, which takes place in Butrinti lagoon (4). This lagoon is located in southern Albania, with an area of 1600 ha and has a production capacity of about 4,500 tons of mussels / year (1).

Vibrios are microorganisms spread in coastal waters around the world and they can contaminate bivalve molluscs. *Vibrio* species are tolerant to salt so they can survive in marine waters and pose a serious threat to public health. They are pathogenic to humans and mortality by members of *Vibrionaceae* family is 10 times higher than that caused by *Salmonella* or *Escherichia coli* (8).

The aim of this study was to assess the presence and incidence of *Vibrio* spp. in bivalve mussels, as very important pathogens causing gastroenteritis in humans.

### Materials and methods

This study was carried out on samples of bivalve mussels of species *Mytilus galloprovincialis* collected from Butrinti lagoon during the period January 2010 - December 2012, by analyzing a total of 234 samples. The samples were taken from 3 stations of the lagoon: North, South and West. All the analyses were performed in the laboratories of microbiological control for aquatic products at Food Safety and Veterinary Institute of Tirana.

Detection of *Vibrio spp.*

Detection of *Vibrio spp.* was performed according to ISO / TS 21872-1:2007 (7). For initial dilution the enrichment media Alkaline Saline Peptone Water (ASPW) was used. The representative sample (25 g) was homogenized with 225 ml ASPW. Further, this homogenate was incubated at 41.5 °C for 6 h ± 1 h. After 24 hours the culture obtained was inoculated with a loop to the surface of the plate with TCBS agar and *Vibrio* CROM agar, in order to allow the growth of well isolated colonies. The presence of opaque yellow colonies with a diameter of 2-3 mm in TCBS was indicative of the presence of *V. cholerae*, *V. alginolyticus*, *V. fluvial* and/or, *V. metschnikovii*, while the growth of green colonies was indicative of the presence of *V. parahaemolyticus*, *V. vulnificus*, *V. harvey* or *V. mimicus*. In *Vibrio* CHROM agar the presence of smooth colonies with blue color indicated the presence of *V. cholerae* and smooth red colonies indicated the presence of *V. parahaemolyticus*. At the end of incubation, suspected colonies were subject to the following tests for confirmation: determination of oxidative activity, planting in tryptophan broth for indole reaction, halotolerance test (inoculation in Peptone Water with different salt concentrations), and API 20E.

### Results and discussion

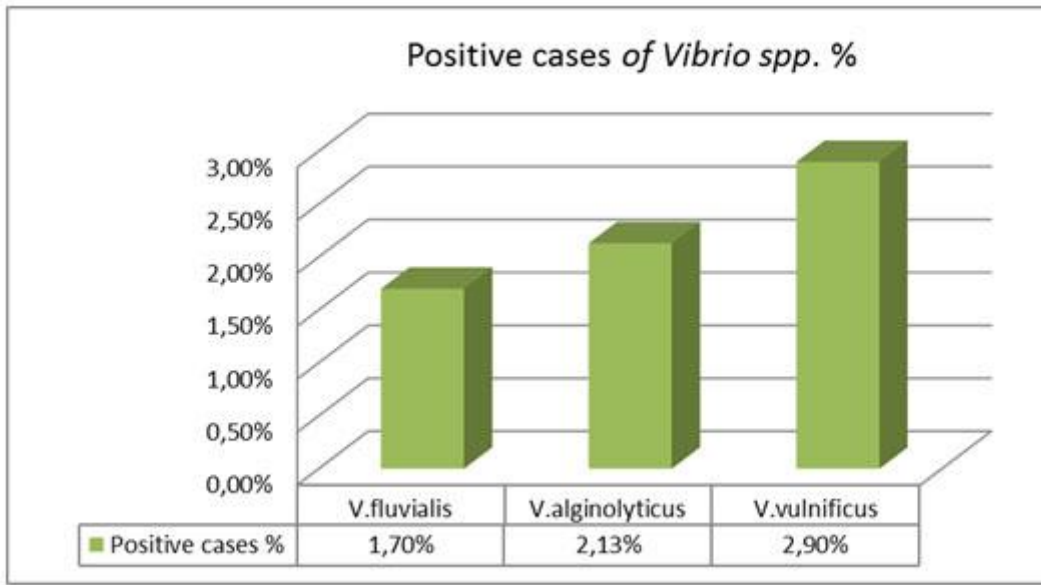
In this study, carried out during the period 2010-2012, the number and prevalence (%) of samples that resulted positive for the presence of *Vibrio spp.* were the following:

- 7 samples with *V. vulnificus* (2.9%);
- 4 samples with *V. fluvialis* (1.7%);
- 5 samples with *V. alginolyticus* (2.13%)

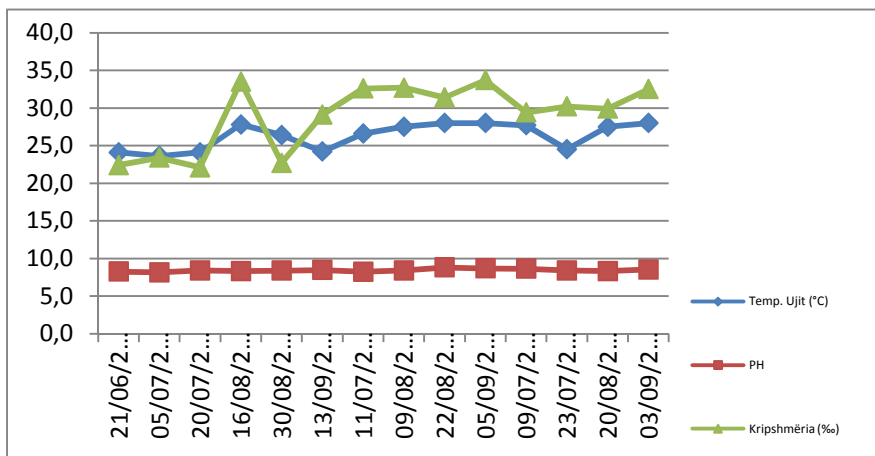
The table below shows the positive results of *Vibrio spp.* as well as the data on physic-chemical indicators of sea water, at the time of sampling.

Date of sampling	<i>Vibrio spp.</i>			Water temp. (°C)	pH	Salinity (‰)
	<i>V. vulnificus</i>	<i>V. fluvialis</i>	<i>V. alginolyticus</i>			
21/06/2010		1	1	24.1	8.24	22.4
05/07/2010	1			23.6	8.15	23.4
20/07/2010	1			24.1	8.39	22.1
16/08/2010		1		27.8	8.32	33.5
30/08/2010		1	1	26.4	8.38	22.7
13/09/2010	1			24.2	8.47	29.1
11/07/2011		1		26.6	8.22	32.6
09/08/2011	1			27.5	8.4	32.7
22/08/2011	1			28	8.8	31.4
05/09/2011	1			28	8.67	33.7
09/07/2012			1	27.7	8.62	29.4
23/07/2012	1			24.5	8.4	30.2
20/08/2012			1	27.5	8.32	29.9
03/09/2012			1	28	8.52	32.5
TOTAL	7	4	5			

Percentage (on a total of 234 samples)	<b>2.9 %</b>	<b>1.70 %</b>	<b>2.13 %</b>			
Maximum				28.00	8.80	33.70
Average				<b>26.2°C</b>	<b>8.42</b>	<b>28.97 ‰</b>



As shown in the table and graph above, out of 234 samples analyzed in a three-year period. All the positive cases of *Vibrio* spp. were isolated between June-September. By analyzing the physical and chemical indicators of water it was observed that the above mentioned species of *Vibrio* were isolated at the average values of 26.2 °C of temperature, 8.42 of pH and 28.97 ‰ of salinity.



Based on the results obtained from this study, *Vibrio* species isolated from Butrinti lagoon molluscs were adapted in physical-chemical conditions of water: temperature between 23.6-28° C, pH between 8.15 to 8.8 and salinity between 22.1-33.7 ‰.

### Conclusions

Out of 234 samples analyzed those positive for the presence of *Vibrio* spp. were as follows: 7 samples positive for *V. vulnificus* (2.9%), 4 for *V. fluvialis* (1.7%), 5 for *V. alginolyticus* (2.13%). *V. vulnificus*, *V. alginolyticus* and *V. fluvialis* are among *Vibrio* species of interest for humans, as they can cause food poisoning (6).

Positive cases of *Vibrio* spp. were isolated during the period June-September, indicating that *Vibrio* species have a preference on high water temperatures (5).

*Vibrio* species isolated from Butrinti lagoon molluscs were adapted in physical-chemical conditions such as water temperature intervals 23.6-28° C, pH 8.15 to 8.8 and salinity 22.1-33.7 ‰.

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## EFFICIENT PEST CONTROL IN OILSEED RAPE AND POSSIBILITIES FOR PROTECTING THE NATURAL POLLINATORS AND HONEY BEES

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### Abstract

Oilseed rape is attacked by a number of pests, which could compromise the yield and quality of the produce. Losses caused by them could be reduced by applying a broad spectrum of pesticides (insecticides and fungicides) that often have a negative effect on the natural pollinators and honey bees.

Studies were carried out in the period 2010-2013 in the Training-and-Experimental Fields of the Agricultural University – Plovdiv and in industrial production areas in the regions of Pazardzhik and Plovdiv.

Phenological development of oilseed rape and the accompanying phytopathological and entomological problems enabled us to develop a plant protection model. The established critical period at the stages of buttoning – flowering – fruit set, includes the choice of applying the plant protection chemical and the time for realizing efficient control, combined with the possibilities for protecting the pollinators of oilseed rape.

**Key words:** oilseed rape, pests, chemical control, pollinators

### Introduction

Oilseed rape is a crop with a long period of vegetation which starts in the beginning of September and lasts until June. This makes it necessary for the plant protection activities to encompass the autumn-spring period. The formation of the rosette in autumn is an important prerequisite for the successful wintering of the crop and its resistance to low temperatures. It has been proven that 50% of the yield is formed in autumn. The formed leaves shall be protected against diseases and pests.

Within the period stretching from the moment of growing to the formation of the rosette, damages are usually caused by the following pests: flea beetles of the *Phyll treta* genus, cabbage stem flea beetles (*Psylliodes chrysocephala* L.), red turnip beetles (*Entomoscelis adonidis* Pall) and coleseed sawflies (*Athalia rosae* L.) (Palagacheva and Dimitrov, 2011). In addition to the pests during the early vegetation period, serious damages are also caused by the fungal diseases blackleg (*Leptosphaeria maculans* (Desm.) Ces.& de Not.; *Phoma lingam* (Tode ex Fr.) Desm) and leaf spots (*Alternaria brassicae* (Berk.) Sacc.) (Encheva-Vasileva, 2006; Yu Chen, 2006; Tonev, 2008; Bankina, 2010).

In the early spring as the temperatures rise, the oilseed rape resumes its growth and forms a flowering stem. Throughout the entire vegetation period, it is inhabited by a large number of harmful and also useful insects. From the rosette phenophase to the stage of buttoning-flowering and fruit set, the following insects are detected: flea beetles of the *Phyll treta* genus, cabbage stem flea beetles (*Psylliodes chrysocephala* L.), pollen beetles - *Meligethes aeneus* F., beetles – *Tropinota hirta* Poda, cabbage stem weevils (*Ceutorhynchus quadridens* Panz.(*C.pallidactylus* Marsh.), cabbage seedpod weevils (*Ceutorhynchus assimilis* Payk.(*C.obstrictus* Marsh.), rape stem weevils (*Ceuthorhynchus napi* L.) (Palagacheva and Dimitrov, 2011). The detected diseases are the following: blackleg (*L. maculans*; *Phoma lingam*), white mold (*S. sclerotiorum*), gray mold rot (*Botrytis cinerea* Persoon), leaf spots (*Alternaria brassicae* (Berk.) Sacc.), powdery mildew

(*Erysiphe cruciferarum* (Opiz ex L. Junell) (Encheva-Vasileva et al., 2006; Yu Chen and Fernando, 2006; Tonev et al., 2008; Bankina et al., 2010).

The protection of oilseed rape against pests is related to the execution of a number of activities which include the efficient completion of the overall fight and the protection of the natural pollinators and the bees as well. In autumn they are used for the purpose of limiting the scope of damage and improving the phytosanitary status of the crops and in the spring they are intended to protect the plants and provide good-quality high yield.

About 80% of the flowering plants are pollinated by bees and other useful types which constitute only 20% of all insects (Naydenov and Zaharinov, 2012). Based on the data provided by Food Agricultural Organization (FAO), out of 100 types of plants which provide 90% of the food worldwide, 71 are pollinated by bees. The effect of pollination is 20-25 times greater than the value of the obtained yield of honey.

Bees play an important role in the pollination of the oilseed rape, as a result of which we obtain a higher yield and good-quality seeds of this crop compared to the case when pollination is performed by the wind (Bommarco et al., 2012). In their studies Abrol et al. (2007) also emphasize the importance of bees among natural pollinators. The yield of rape and mustard seeds is doubled when pollination is done by the insects. Pollinators not only increase the yield of the crop but also contribute to the even and early formation of the pods.

The increased use of pesticides over the last few years significantly raised the mortality rate of the natural pollinators and the bees. In their studies Gill et al. (2012) have established that the use of neonicotinoids and pyrethroids has a negative influence on the large earth bumblebees, increases the mortality rate of the worker bees and considerably reduces the number of the offspring, which eventually leads to the death of the family. Neonicotinoids also affect the behaviour of the bees (Fischer et al., 2013). According to Henry et al. (2012), the contact with insignificant quantities of tiametoxam causes disorientation and inability of the bees to find the beehives.

The “empty beehive” syndrome and the introduced changes in the European legislation restricting the application of some neonicotinoids provide conditions for establishing plant protection models aimed at ensuring the effective protection of oilseed rape, the natural pollinators and the bees, which is the purpose of this survey.

### **Material and methods**

The studies were conducted within the period 2010-2013 in the Training-and-Experimental Fields of the Agricultural University – Plovdiv and in the industrial production areas in the regions of Pazardzhik and Plovdiv. We conducted systemic observations on the emergence and the development of pests and diseases on the oilseed rape. The registering was implemented on the grounds of the common entomological and phytopathological methods – using an entomological net, sample lots, observations and visual inspections of the plants.

The index of diseases attack has been calculated using the formula of McKinney (Josefovich, 1956). The identification of the agents was performed using a macroscopic and a microscopic analysis.

The treatments of the crops were performed using preparations from the groups of the organophosphorus insecticides, the synthetic pyrethroids and oxadiazins. Their efficiency was observed by the 15<sup>th</sup> day after treatment.

### **Results and discussion**

The conducted survey shows that the first pests in the rape agrocenosis during the cotyledon – rosette formation phenophase are the black cabbage flea beetles (*Phyllotreta atra* F.), the striped cabbage flea beetles (*Phyllotreta undulata* Kutsch.) and the cabbage flea beetles (*Psylliodes chrysocephala* L.). They are found in a mixed population. Out of all aforementioned types, the

cabbage stem flea beetle was proven to have the largest population – 2-3 insects/m<sup>2</sup> (Figure 1). The emergence of the adult insects in autumn coincided with the growing of the rape plants. The black cabbage flea beetle had permanent density during the three years of the experiment – 2 insects/m<sup>2</sup>. Single insects of the striped cabbage flea beetles were found (1insect/m<sup>2</sup>). Low numbers of red turnip beetles (*Entomoscelis adonidis* Pall.) were detected in some areas planted with the crop around the city of Plovdiv. The coleseed sawfly (*Athalia rosae* L.) appeared in the periphery of the lots in small areas, especially in the region around the city of Plovdiv.

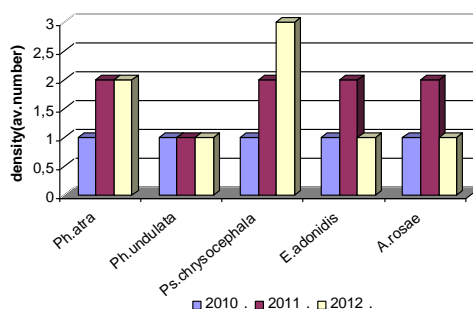


Figure 1. Species composition and density (insects/m<sup>2</sup>) of the harmful entomofauna in the rape agrocenosis in autumn.

The first symptoms of the economically significant disease blackleg (*Phoma lingam*) over the years of observation were manifested at the end of October and in the beginning of November in the form of rounded white to grey spots with black pycnidia. The index of the disease attack ranged from 10,5% to 16,3% (Figure 2).

The resumption of the rape development in the spring started as the temperature rose to over 5 °C. The attack of the fungal disease blackleg was registered in the second ten days of April as the degree of development was 12,7 % in 2011, 17,5 % in 2012 and 35,5 % in 2013. In 2013 in the beginning of the vegetation period, we registered frequent rainfalls, which is a prerequisite for the more widespread development of the blackleg. During the second ten days of May, powdery mildew (*Erysiphe cruciferarum*) was detected on some individual plants in the form of spots with off-white powdery coating.

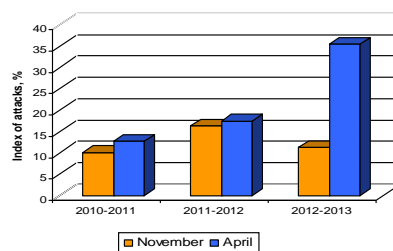


Figure 2. Index of the blackleg attack (*Phoma lingam*) on oilseed rape.

The first pests after the rise of the temperatures in the rape agrocenosis were the flea beetles and the cabbage stem flea beetles. Low density was registered - 1-2 insects/m<sup>2</sup>. During the second half of April, with the rise of the temperatures we detected single insects of red cabbage bugs: the red cabbage bug (*Eurydema ornata* L.) and cabbage shield bug (*Eurydema oleraceum* L.). In some plants their density reached 1-2 insects/m<sup>2</sup> (Figure 3).

With the beginning of the buttoning and flowering stages, the following pests were detected: the pollen beetles (*Meligethes aeneus* F.), the beetles (*Tropinota hirta* Poda), the white-spotted rose beetles (*Oxytirea funesta* Poda). The type with the greatest economic importance is the pollen beetle

whose density was found to be the highest 3-7 insects/m<sup>2</sup>. Out of the weevils, we detected the following types: cabbage stem weevils (*Ceutorhynchus quadridens* Panz. (*C. pallidactylus* Marsh.), rape stem weevils (*Ceutorhynchus napi* L.) and cabbage seedpod weevils (*Ceutorhynchus obstrictus* Marsh. (*C. assimilis*). We also found caterpillars of the large white butterfly (*Pieris brassicae* L.) and the small white butterfly (*Pieris rapae* L.). When the pods ripened as a result of the increase in humidity, even if the density was low (1-2 grades), the cabbage aphid (*Brevicoryne brassicae* L.) appeared annually.

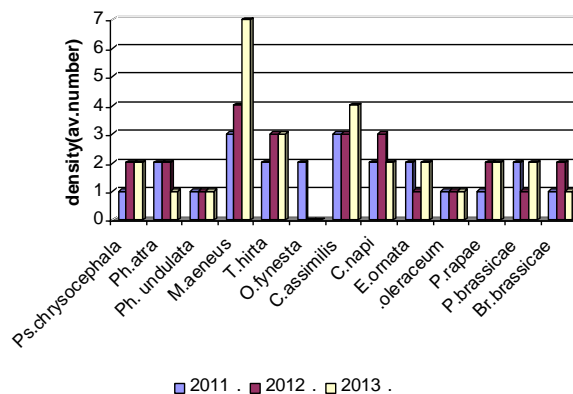


Figure 3. Species composition and density (insects/m<sup>2</sup>) of the harmful entomofauna in the rape agrocenosis in spring.

Regarding the established entomofauna, the following types belong to the *Coleoptera* genus: flea beetles, cabbage stem flea beetles, red turnip beetles, pollen beetles, the weevils of the *Ceutorhynchus* genus, alleculid beetles and white-spotted rose beetles. The insects belonging to the *Hemiptera* genus, *Homoptera* subgenus, are the cabbage aphids; the insects belonging to the *Heteroptera* subgenus are the red cabbage bugs; the insects belonging to the *Hymenoptera* genus are the coleseed sawflies; the insects that belong to the *Lepidoptera* genus are the white cabbage butterflies and the small white butterflies.

The established species composition has a strict organotrophic specialization. The leaves feed the following insects: flea beetles, cabbage stem flea beetles, red turnip beetles, coleseed sawflies, red cabbage bugs, white cabbage butterflies, small white butterflies, cabbage aphids; the stem feeds the following insects: cabbage stem flea beetles, cabbage stem weevils, rape stem weevils; the flowers feed the following insects: pollen beetles, alleculid beetles, white-spotted rose beetles and the pods feed the cabbage seedpod weevils.

The dynamics of the population of the types results from the existing weather conditions and the phenological development of the oilseed rape (Figure 4).

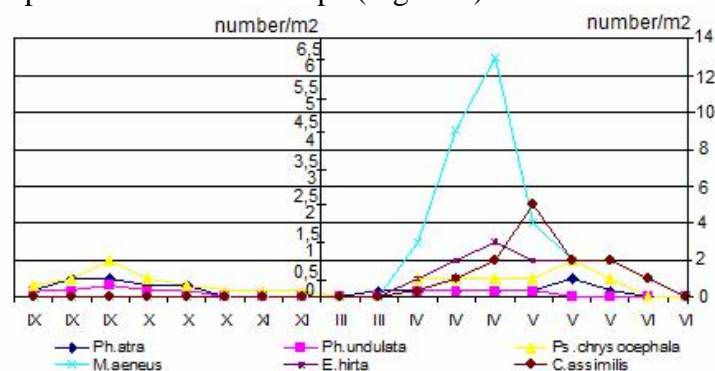


Figure 4. Dynamics of the density of the population of the main pests in the rape agrocenosis within the period 2010-2013.

During the autumn-winter period, the flea beetles of the *Phyllotreta* genus and the cabbage stem flea beetles dominated. The highest density was registered for the cabbage stem flea beetle – 1 insect/m<sup>2</sup>. Its development lasted until November. The cabbage flea beetles *Ph. atra* and *Ph. undulata* had a smaller population as the highest values were registered at the end of September.

In the spring as the temperatures rose to over 5 °C, the oilseed rape started developing and the harmful and useful entomofauna started reproducing. The fleas appeared in March and their development lasted until June. Their number was the largest during the second ten days of May.

With the start of the buttoning phenophase, the density of the population of the pollen beetles dramatically increased. The type reached its peak of 13 insects/m<sup>2</sup> during the third ten days of April, which coincided with the emergence of the cabbage seedpod weevils. The wide spread of the weevils in the rape fields was detected during the mass flowering stage. The parallel development of both species resulted in competition for food and habitat, which caused the migration of the pollen beetles outside the planted areas.

In the beginning of April, the alleculid beetles appeared in the rape fields. The number of this species reached 1-2 insect/m<sup>2</sup> as the peak value of the density of the population was registered at the end of April.

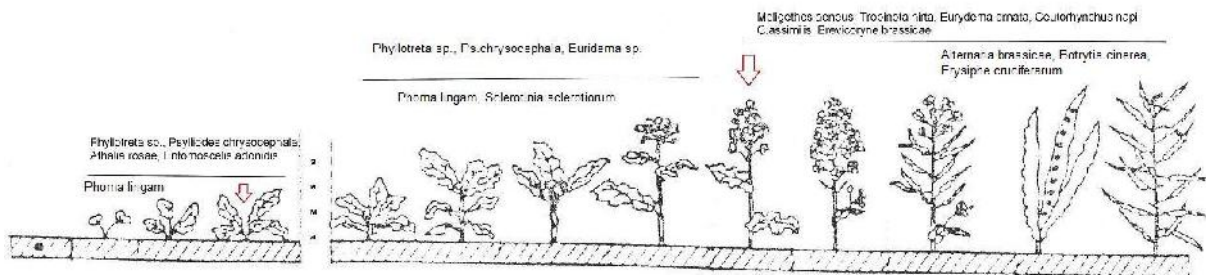


Figure 5. More important pests in the rape agrocenosis and appropriate periods of time to fight them

The fight against the pests on the rape plants starts in autumn when reaching the critical threshold of economic harmfulness: 2 insects/m<sup>2</sup> for the cabbage stem flea beetles, 2-3 insects/m<sup>2</sup> for the coleseed sawflies and 3-5 insects/m<sup>2</sup> for the flea beetles. The end of the calendar year and the forthcoming winter allow us to use preparations with a wider scope of activity and a long-term effect, such as: organophosphorus insecticides, (active substance chlorpyrifos-ethyl), synthetic pyrethroids (deltamethrin, alpha-cypermethrin, lambda cyhalothrin, cypermethrin + chlorpyrifos-ethyl, tau-fluvalinate) and neonicotinoids (active substance thiacloprid, thiacloprid + deltamethrin, Figure 5, Table 1).

In November and October there were favourable conditions (positive temperatures and high humidity) for the emergence and development of blackleg, leaf spots and powdery mildew. The systemic triazole fungicides show good effectiveness (active substance metkonazol, tebuconazole) (Table 1). When using them, they not only protect the crop from diseases, but also regulate the growth of the plants and prevent the risk of overgrowth.

In the spring, for the purpose of protecting the rape against blackleg, leaf spots, white mold, gray mold rot, powdery mildew and also for enhancing the resistance of plants against leaning, we apply treatment using the fungicides Caramba 60 EC (metkonazol) when the height of the plants reaches 23-35 cm and Folicur 250 EW (tebuconazole) during the buttoning phenophase. Under the conditions of frequent rainfalls and high relative humidity in the beginning of the flowering stage, fungicidal treatment shall be applied using Pictor SC (dimoxystrobin + boscalid) or Prosaro 250 EC (prothioconazole+ tebuconazole) in order to prevent the development of the white mold and the

gray mold rot, the blackleg and the leaf spots. These preparations are completely harmless for the bees when applied in accordance with their registration.

The successful completion of the fight against the pests on the rape during the spring is the factor that predetermines to the largest extent the successful protection of the cultivated crop and the useful entomofauna and also the pollinators in the rape agrocenosis. Among all other necessary activities, an important role is played by the monitoring approach that registers the dynamics in the density of the population.

Table 1 Approved pesticides for fighting pests in the rape agrocenosis

<b>INSECTICIDE</b>	<b>ACTIVE SUBSTANCE</b>	<b>CHEMICAL GROUP</b>
AVANT 150 EC	indoxacarb	oxadiazin
BISCAYA 240 OD	thiacloprid	neonicotinoid
PROTEUS 110 OD	thiacloprid + deltamethrin	neonicotinoid
CALYPSO 480 SC	thiacloprid	neonicotinoid
DEKA EK	deltametrin	synthetic pyrethroid
FASTAC NOV	alpha-cypermethrin	synthetic pyrethroid
KARATE ZEON	lambda cyhalothrin	synthetic pyrethroid
NURELLE D	cypermethrin+chlorpyrifos-ethyl	synthetic pyrethroid
MAVRIK 2F	tau-fluvalinate	synthetic pyrethroid
DURSBAN 4E	chlorpyrifos-ethyl	organophosphate
<b>FUNGICIDE</b>	<b>ACTIVE SUBSTANCE</b>	<b>CHEMICAL GROUP</b>
CARAMBA 60 EC	metkonazol	Triazole fungicide and growth regulator
ORIUS 25 EW	tebuconazole	Triazole fungicide
PICTOR SC	dimoxystrobin + boscalid	Two-component systemic fungicide
PROSARO 250 EC	prothioconazole+ tebuconazole	Triazole fungicide
FOLICUR 250 EW HORIZON	tebuconazole	Triazole fungicide

The weed known as wild mustard (*Sinapis arvensis*), which is suitable to be a host for flea beetles, aphides and pollen beetles, is regarded as an indicator of the phytosanitary status of the region. When the number of the pollen beetle is 2-4 insects/plant, there is a serious risk of its reproduction during the vegetation period, which necessitates conducting weekly examinations of the crops. After the examinations, it was established that the attack starts from the periphery (the pest chooses plants of a higher habitus) towards the inner areas of the crops and is related to the initial formation of buds. When the number reaches 1-2 insects/m for 15-20% of the attacked plants, it is necessary to apply treatment to the peripheral area of the crops that is 10-12 m wide, using preparations based on chlorpyrifos-ethyl. This enables us to limit the migration of the pollen beetle towards the inner area of the crops and also to preserve the natural pollinators and the bees whose density is still limited.

If it is necessary to apply a second treatment (during the “green-button” phenophase), we can use formulations that ensure high effectiveness for 5-6 days and have a repellent effect for about 7-8 days. This is the capacity of the insecticide Avant - 17 ml/dka (it belongs to the group of oxadiazins) in combination with the adhesive Codacide - 200 ml/dka.

In case of high density of the cabbage seedpod weevils and reproduction of the pollen beetles at the end of the flowering stage and the formation of the pods, the treatment is conducted using the preparation Biscaya, whose tolerance towards pollinators and bees has been proven.

## Conclusion

As a result of the conducted survey, we can draw the following conclusions:

- The rape acts as a host for a number of diseases and pests. In autumn it is attacked by the following pests: the flea beetles of the *Phyll treta* genus, the cabbage stem flea beetles (*Psylliodes chrysocephala* L.), the red turnip beetles (*Entomoscelis adonidis* Pall.) and the coleseed sawflies (*Athalia rosae* L.) and also by the following diseases: blackleg (dry stem rot) (*Leptosphaeria maculans* (Desm.) Ces.& de Not.; *Phoma lingam* (Tode ex Fr.) Desm) and leaf spots (*Alternaria brassicae* (Berk.) Sacc.). In the spring, the following pests develop on its plants: flea beetles of the *Phyll treta* genus, the cabbage stem flea beetles (*Psylliodes chrysocephala* L.), the pollen beetles - *Meligethes aeneus* F., the beetles – *Tropinota hirta* Poda, the cabbage stem weevils (*Ceutorhynchus quadridens* Panz.), the cabbage seedpod weevils (*Ceutorhynchus assimilis* Payk.(*C.obstrictus* Marsh.), the rape stem weevils (*Ceuthorhynchus napi* L.). The diseases detected are: blackleg (*L. maculans*; *Phoma lingam*), white mold (*S. sclerotiorum*), gray mold rot (*Botrytis cinerea* Persoon), leaf spots (*Alternaria brassicae* (Berk.) Sacc.) and powdery mildew (*Erysiphe cruciferarum* (Opiz ex L. Junell).

- During the vegetation of the winter rape there are two critical periods as a result of the pest attacks – an autumn period covering the second half of September and the beginning of October when the plants are attacked by the flea beetles and the blackleg and a spring period – April (lengthening of the stem – buttoning) for the development of the blackleg, the white mold and the reproduction of the pollen beetles.

- The protection of the pollinators and the honey bees requires the application of minimal treatment of the crops that is related to the exact prognosis regarding the emergence and the development of the pollen beetle. By applying treatment to an area 10-12 m wide, using preparations based on chlorpyrifos-ethyl and if necessary treating the crops with the insecticide Avant 17ml/dka and the adhesive Codacide -200 ml/dka during the “green-button” stage, we can ensure an effective fight against the pollen beetle which has the slightest negative effect on the natural pollinators and the bees.

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## AGRICULTURAL PRODUCTION CONTRACTS AND FOOD SAFETY

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### Abstract

Food safety is currently one of the most urgent issues in both EU's and global agriculture. One of its basis tool might be the cultivation contract that is strictly related with the process of production and supply in agriculture.

The contract in question has numerous functions, which are modified along with the technological progress. However, it should still be seen as the main stem of the system supplying raw materials for the agri-food industry. The contract plays a huge role in the agro-logistics chain. It covers such elements as planning, gathering, controlling and flow of the materials that are directed to the processing or sell. Besides, it should be perceive as a legal instrument limiting broadly understood risk in agricultural production.

The aim of the article is an attempt to indicate the role of the production contract in providing the food safety. The article also aims to answer the question what safety issues might be covered by such a contract to fulfill the general safety standards.

**Key words:** agricultural production contracts, production risk, food safety, agri-food industry, food chain.

### Introduction

An agricultural production contract is a contract by which a producer (“grower”) agrees to produce and deliver all of a designated crop raised or agrees to feed and care for livestock in a manner set forth in the agreement to a contractor (quite often processor). The producer is paid according to a formula established in the contract. Such a contract usually specifies in detail the production inputs to be supplied by the contractor, the quality and quantity of the particular commodity involved, the production practices to be used, and the manner in which compensation is to be paid to the producer. The Polish Civil Code defines contract farming as “a farmer commitment to produce and supply to the contracting party a specified quantity of agricultural produce of a given time, and the contracting party commits to collect the produce at an agreed time, pay the agreed price and make a specified additional performance if the contract or specific regulation provide for the obligation to make such performance<sup>4</sup>. The quantity of agricultural produce may also be specified in the contract according to the area from which the produce is to be gathered (Article No 613 of Polish Civil Code, 1964).

Due to remarkable process of transformation in the agri-food sectors, contracts are an increasingly important aspect of agricultural production. They raise concerns about market transparency, possible consequences for contract's parties of the food supply chains and the role of a legislator and government in the changing environment (Wu, 2003). Thus it is very important to determine the “nature” of production contract, as the food safety instrument, that will be comprehensible, concise and possible to fulfill by both parties – the agricultural producers and the processors.

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<sup>4</sup> A farmer also means a group or association of farmers.

It is safe to assume that the agricultural production is no more the autonomous production. It is covered by multi layers structure, called coordination. The contract coordination from the farm through the processor to the consumer is intended to ensure that quality and other product specifications are met. Quality incentives for various players along the vertical chain often are needed, and technology transfer (e.g., specialized inputs, buildings) by processors to use by growers may occur. Therefore it is important to separate the food safety notion from both farmer and processor/consumer point of view.

### **Materials and methods**

The objective of this paper is to indicate the role of the production contract in providing the food safety. The article also aims to answer the question what safety issues might be covered by such a contract to fulfill the general safety standards.

This objective has been achieved in two stages. The first step was to identify and analyze the legal definition of food safety. That was based on the European Union's and Polish legislation and followed by brief legal characteristic of contract nature based on Polish Civil Code. Because of the wide scope of contracts' significance, only some of them were chosen to further research. Next, taking into consideration that the production contract has fulfilled the assumptions of the concept of food safety, the contract's impact on the selected stages in agri-food chain have been presented according to this concept. The analysis of theoretical approaches based on literature has been enriched with legal and economic literature and the observations made by the author. The main methods applied in the article were the dogmatic analysis of the EU and national regulations as well as the descriptive method.

### **Results and discussion**

Food safety is a flexible concept, reflected in many attempts at definition in research and policy usage. It might be understood as scientific discipline describing handling, preparation, and storage of food in ways that prevent foodborne illness.

Food safety is the term that has its own legal definition. Although the legislator has not determined it directly, in positive sense. He has framed it in reverse. According to Article 14 of Reg. No 178/2002 food shall be deemed to be unsafe if it is considered to be injurious to health and unfit for human consumption (Guidance, 2010) Such food shall not be placed on the market. The safety and acceptability of food is of critical importance. Consumers must have confidence and assurance that the food they buy will be what they expect and will do them no harm or have an adverse effect. The aim of Article 14 is to protect the consumer from food that is either a health risk or unacceptable. The mentioned article defines the general food safety requirements which are used with the risk management requirements covered by Article 19 to reduce or eliminate any risk due to the placing of unsafe foods on the market. The definition of 'placing on the market' is quite wide and it includes all sales and supplies, including one-off sales, one-off supplies free of charge, and holding food for the purpose of sale. The Article does not, however, cover primary production for private domestic use, or the use of food for private domestic consumption, which are exempted by the Regulation No 178/2002.

The free movement of safe and wholesome food is an essential aspect of the internal market and contributes significantly to the health and well-being of citizens, and to their social and economic interests (recital 1 of Reg. No 178/2002). Thus it was necessary to adopt measures aimed at guaranteeing that unsafe food is not placed on the market and at ensuring that systems exist to identify and respond to food safety problems in order to ensure the proper functioning of the internal market. Similar issues relating to feed safety are addressed. Beside the general EU's rules,

some member states have adopted horizontal legislation on food safety imposing, in particular, a general obligation on economic operators to market only food that is safe.

For example, Polish definition of food safety differs from the EU's. On the basis of Article No 3 of Act of August 25<sup>th</sup> 2006 - Food and Feed Safety<sup>5</sup>, the food safety must be understood as the general conditions which have to be met and considered, such as: food additives, contaminations, remains of pesticides, irradiate conditions, organoleptic features and all activities that should be undertaken on all production's stages. Their aim is to assure humans wellness.

However, member states apply different basic criteria for establishing whether a food is safe. Given these different approaches, and in the absence of horizontal legislation in other member states, barriers to trade in foods are liable to arise. Similarly such barriers may arise to trade in feed. Therefore it is necessary to establish general requirements for only safe food and feed to be placed on the market, to ensure that the internal market in such products functions effectively on the basis of production contracts.

The food safety has also the economic dimension. It considers the food security as a matter of raw materials supply and is tied to one of the contracts' functions. The production contract is strictly related with two production stages – the production and delivery of the agricultural product. The contract secures the volume of production specified in the contract. For the agricultural producer it is a guarantee of the possibility to make a previously agreed amount of products, e.g. according to the mass or yield from a particular area. Thus, the farmer has a guarantee of the market and payment for the products.

As food safety and the food security, in the final market depend on several stages of the supply chain. Some kind of vertical coordination is necessary in order to assure compliance with regulations and to avoid potentially negative demand effects. Vertical coordination refers to the synchronization of successive stages of production and marketing, with respect to quantity, quality, and timing of product flows (Arzu et al., 2006). That is why coordination is achieved by written contracts regulating the relationship between farmers and contractors.

The use of formalized written contracts concluded in advance of production and delivery containing basic elements is not widespread. It is vivid on selected markets, like sugar, fruits and vegetables, swine, rapes, etc. It always takes a place wherever it is necessary to secure a certain volume of production. But it might be the best way to assure the quality standards. It guarantees that the agricultural producer will supply agricultural products of the required not only quantity but also quality. It is not only a matter of achieving the desirable parameters of the supplied raw material, but also a matter of production from the input material indicated by the contracting authority, usually according to their cultivation recommendations (Lipi ska, 2013).

Contracts play a huge role in a production chain, because they not only ensure quality but they guarantee some other product specifications. Quality incentives for some players along the vertical chain often are needed. On a basis of the contracts the new technology (e.g., specialized inputs, buildings) might be transferred by processors to farmers. The same time they may casted their use, otherwise the contractor might cancel the goods collection as well as terminate the contract on farmer's fault.

However there great disparity might be seen in bargaining power and marketing information between contractor (e.g., processors or integrators) and farmers. Thus EU's policy makers have responded with Regulation (EU) No 261/2012 of the European Parliament and of the Council of 14 March 2012 amending Council Regulation (EC) No 1234/2007 as regards contractual relations in the milk and milk products sector (O.J. UE L 94, p. 38-48). The legislation regulates only selected agricultural contracts, but it might be the milestone in covering other agricultural markets and sectors. In this case, among the basic conditions, it is important that the price payable for the delivery can be set in the contract, at the choice of the contracting parties, as a static price or a price

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<sup>5</sup>O.J. 2010, No 136, 914 with subsequent amendments.

varying depending on defined factors. For example, on the basis of Article No Point 7 Council Regulation No 261/2012 the price for milk shall be depended on the volume and the quality or composition of the raw milk delivered.

In the absence of Union legislation concerning production contracts, the legislator expressed that member states may, within their own contract law systems, decide to make the use of such contracts compulsory provided that in doing so Union law is respected and in particular that the proper functioning of the internal market and the common market organization is respected. In view of the diversity of the situations that exist across the Union in relation to contract law, in the interests of subsidiarity, such a decision should remain with Member States<sup>6</sup>.

In the environment of industrialized and modern agriculture setting the legal model of production contract is like a puzzle for lawmakers and regulators (Wu, 2003). On the one hand, the greater coordination of the food system through such contracts and integration has efficiency, equitability, and power effects. On the other hand, the increased concentration of large processors is eroding the economic power of small growers, raising questions about the equitability of contract arrangements, and expanding the potential for fraud and other dishonest behavior. And no one should forget that that agricultural food safety involves the primary production and processing of raw agricultural products, and it starts from the very beginning of food supply chain.

In order to ensure appropriate minimum standards for such contracts and to ensure that the internal market and the common market organization function well according to the food safety, some basic conditions for the use of such contracts should be laid down at least domestic and even Union level. All such basic conditions should, however, be freely negotiated. One of its example is vivid in Polish Civil Code and French Code Rural (Code,2013).

The new agricultural economy is characterized by two main overriding features, such as greater concentration (intensity) of farms into smaller numbers with large sizes and rising influence of contract farming, and the evolution of integrated supply chains linking producers and consumers (Opara, 2003). Such intensive agriculture relies heavily on irrigation, agro-chemicals, use of new technologies, and basic raw materials that possess new traits such as genetically modified plants and animals. Under the new agricultural economy, these attributes of intensive farming create new challenges for sustainable production and processing practices that promote a balanced approach to the problems of food quality, safety, and good environmental stewardship. The shift from quantity-oriented agriculture to new emphasis on quality, safety, functionality and sustainability, have placed new demands for the development and adoption of traceable supply chains. Besides contract farming more often implies shorter, direct chains between farmers and companies. This is often essential for traceability and quality upgrading.

In order to ensure large and consistent volumes of high-quality and safe produce, food processors procure from preferred suppliers, on a production contract basis, and thereby push the food distribution system towards more and more vertical integration. Some of them are large scale, and let to increase the scope for standardized production and for meeting high standards at low transaction costs (Maertens &, Swinnen, 2008). Moreover, well-specified contracts include farm extension and assistance programs that can alleviate the financial and technical constraints small farmers in meeting increasingly stringent standards.

Agricultural production and investments always involve risk. Thus one of the main functions of the production contract should be protection of agricultural producers from unfavorable consequences which may appear during the execution of the contract. Agricultural activity is considerably exposed to the risk of external factors, which are usually beyond the farmer's control and which cannot be prevented. This concerns unpredictable natural factors. The most likely reasons for the failure are poor crop management, natural calamities, pest epidemics, market collapse and price fluctuations. The cultivation contract gives a possibility to share the risk of production according to

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<sup>6</sup> See point No. 9 of Regulation 261/2012.

the shared. The aforementioned negative external factors determine the yield volume and influence the price level on the market. By signing a cultivation contract the agricultural producer cannot prevent them directly, but the farmer has a possibility to receive the agreed price regardless of the turmoil on the market. The standard agribusiness approach to indemnify against quantity shortfalls is crop insurance. Its obligation might be one of the production contract provisions. As the farming involved in a contract arrangement becomes technologically more advanced, the range of risks to which it is subject generally becomes more expensive. But in some way this is a “production” risk, that farmer may incur without the proper farm management.

### Conclusion

The production contract regulations are likely to impact the value chain from the farm gate to the consumer through the range and prices of “safe” foods products.

The production contracts may help to reinforce the responsibility of operators in the food chain and increase awareness of the need to better take into account the signals of the market, to improve price transmission and to adapt supply to demand, as well as to help to avoid certain unfair commercial practices.

Contract production may improve the efficiency of the agricultural system and allow a clearer transmission of consumer preferences, and spawned new value-added products for consumers.

From an economics point of view, regulations are intended to correct market failures and/or reduce transaction costs of market participants, as well as assure the food safety.

Contracting is important because it can provide alternative governance mechanisms for the agricultural sector and can often improve the efficiency of supply chains. Improvements can be attributed to an altering of incentives for market participants, to greater co-ordination between the different stages of the chain, as well as managing product quality.

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**CHEMICAL CONTROL OF *CURCULIO NUCUM* L. (COLEOPTERA: CURCULIONIDAE) WITH KNAPSACK SPRAYERS EQUIPPED WITH AIR-ASSISTED ROTARY DISC NOZZLES**

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**Abstract**

The hazelnut (*Corylus avellena* L) is a hard-shelled fruit that is extensively cultivated in The Black Sea Region, Turkey. Turkey is the main producer of hazelnuts in the world, with an average annual production of 600-650 thousand tons, which represents nearly 70% of worldwide production. The hazelnut is one of Turkey's most important agricultural exports.

The nut weevil *Curculio nucum* L. is the most dangerous pest of hazelnut trees. If unchecked, the nut weevil can significantly and adversely affect hazelnut yields. In this study, we used knapsack sprayers with air-assisted rotary disc nozzles for the chemical control of the nut weevil in hazelnut orchards. The study was conducted in the province of Samsun between 2008 and 2009, in an orchard with planting distances of 4 x 4.5 m between the trees, and an average tree height of 4.5-5 m. During the study, insecticide applications were performed at different doses. Important pulverization characteristics (volume median diameter, residue etc.) were determined by performing trace residue studies. The food dye, tartrazine, was used in trace residue studies. Biological effectiveness studies were also performed by using Carbosulfan insecticide.

During the studies, the hazelnut trees were divided horizontally (lower, middle, upper) and vertically (external, middle, central) into different zones, and the consistency of insecticide distribution, insecticide penetration, residue and pesticide loss were determined. It was observed that the residue varied depending on different zones of the hazelnut trees. Based on the trace residue studies, the highest quantities of residue were identified in the middle and lower zones of trees. Biological effectiveness studies were conducted by performing cage and parcel tests. Based on these tests, the number of dead, alive, and paralyzed mature nut weevils was determined. During the study, the insecticide was applied at 1/1 dose, ¾ dose, ½ dose, and at a standard dose of 20 l/da. The biological effectiveness value in the cage studies was 97.34% at full dose, 93.55% at ¾ dose, and 90.67% at ½ dose. The biological effectiveness value in the parcel studies was 97.25% at 1/1 dose, 91.03% at ¾ dose, and 80.27% at ½ dose.

Based on the results of the study, it can be concluded that effective chemical control against nut weevils can be achieved with insecticide applications performed at 1/1 dose, at ¾ dose, and at a standard dose of 20 l/da.

**Keywords:** Hazelnut, *Curculio nucum*, sprayer, residue, biological effectiveness

**Introduction**

It is known from historical records that the cultivation and production of hazelnuts in northern Turkey on the shores of the Black Sea began 2300 years ago. It is also known that Turkey has exported hazelnut for the past six centuries (Duran, 2007).

The hazelnut is one of Turkey's most important agricultural exports. Hazelnut cultivation in Turkey is currently performed over an area of 640,000 hectares and provides an annual yield of 600-650

thousand tons; this represents 70% of worldwide production (Anonymous, 2010a). Turkey exports nearly 90% of the hazelnut that it produces.

In Turkey, the most preferred method for the control of diseases, pests and weeds in hazelnut orchards is chemical control. Depending on the size and structure of their orchards, farmers in Turkey generally use knapsack sprayers for chemical control.

In all stages of production, any disease, pests and weeds that affect the hazelnut tree can decrease the yield and quality of hazelnuts. Among 150 species of insects that are found in hazelnut orchards, the most important is the nut weevil (Tuncer et al., 2002a). In the absence of control measures, the loss of yield caused by *C. nucum* can reach up 30 to 40% (Ali Niaze, 1998).

Until now, no methods other than chemical control have been utilized in Turkey for the control of nut weevils. In addition, producers tend to use excessive doses of insecticides to achieve biological effectiveness with this control method (Tuncer et al., 2001 and 2002b).

Due to the small size of orchards and their location in areas that are generally inclined, various problems are encountered in hazelnut orchards during the application of chemical control methods. These problems include insecticides not reaching target surfaces, and low biological effectiveness. The fact that 91% of the area within the Ordu province in the Black Sea region has more than 12% incline underlines the necessity and importance of developing different insecticide application techniques for hazelnut cultivation.

### Materials and Methods

Cage and plot studies for trace residues and biological effectiveness were organized in the Terme and Atakum counties of the Samsun province in 2008 and 2009. The study was performed in orchards with a planting distance of 4 x 4.5 m between the trees, and an average tree height of 4.5-5 m. The study materials that were used during the study period included knapsack sprayers with air-assisted rotary disc nozzles (Fig. 1), hazelnut orchards, nut weevils, net cages, spectrophotometers, anemometer, tartrazine, laser droplet size measurement device, water sensitive papers (WSP), insecticide Carbosulfan, leaf area meter, water distillation device, blankets, pallets and fixers.

This study was conducted by using knapsack sprayers, cold fogging machines and knapsack sprayers with air-assisted rotary disc nozzles.

Volumetric Mean Diameter (VMD) value was measured by using a Malvern brand Spraytec model device.



Figure 1. Air-assisted rotary disc nozzle



### Trace residue studies

Testing for trace residues was performed by using the food dye tartrazine instead of an insecticide. In this study, the amount of trace residues obtained from the hazelnut trees, the penetration within the hazelnut trees, and the insecticide loss were investigated. The colorimetric method was used in this study (Çilingir, 1983; Özmerzi and Çilingir, 1992). For this purpose the hazelnut trees were divided into three vertical (height) zones and three horizontal (crown width) zones, thus yielding a total of nine sampling zones (Dursun et al., 2008) (Fig. 2). Application at standard value of 20 l/da was performed with knapsack sprayer equipped with an air-assisted rotary disc nozzle.

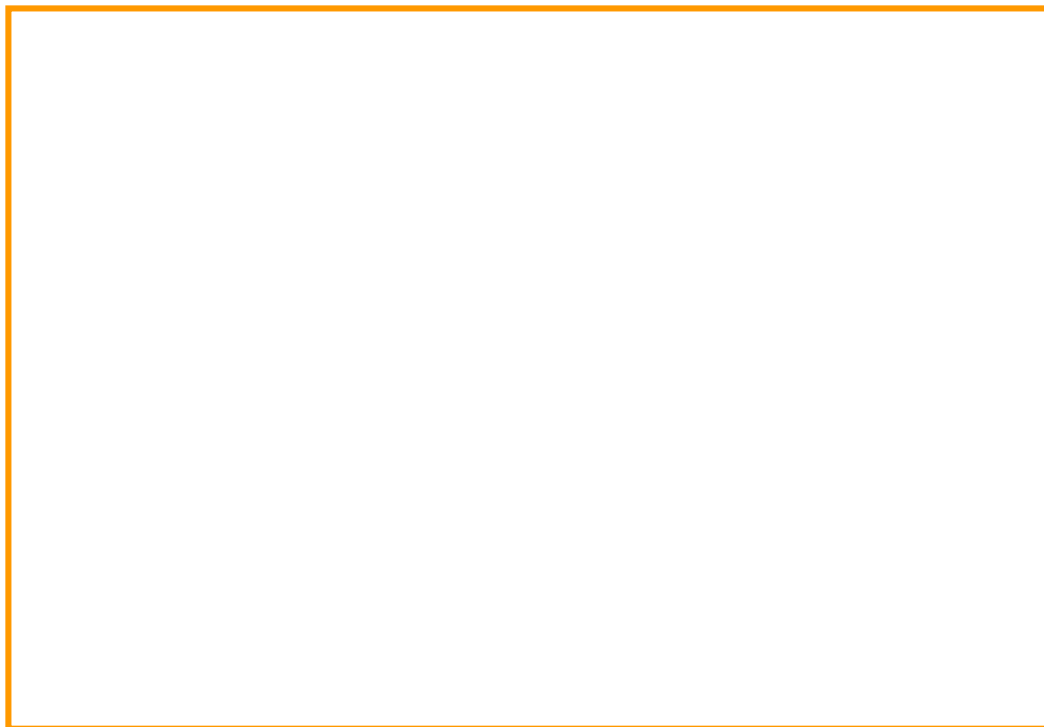


Figure 2. Sampling zones

### Biological effectiveness studies

Biological effectiveness of insecticide studies was conducted between 2008 and 2009 within the context of the cage and parcel studies. The insecticide was applied after most of fruits reached the size of a lentil (3-4 mm). Based on the number of mature nut weevils counted on Day 7 (after insecticide application) during the cage studies performed in 2008 and 2009. Counting paralyzed, dead or alive insects was performed according to Anonymous (1995).

Prior to spraying, mature nut weevils were collected for the cage studies. In order to collect mature adults, 3x4 m blankets made of white cloth were used. The data obtained from the cage studies were evaluated by using the Abbott formula (Karman, 1971). All data were analyzed by analysis of variance JMP Analysis Programme.

### Results and discussion

The  $D_v(50)$  Volumetric Mean Diameter (VMD) value was measured as 64.68  $\mu\text{m}$  by using a Malvern brand Spraytec model device.

The highest quantity of residue was found in the middle external zone with a value of 1.427  $\mu\text{g}/\text{cm}^2$ , while the lowest in the upper central zone with a value of 0.578  $\mu\text{g}/\text{cm}^2$ . The quantity of residue found in the middle and central zones gradually decreased from the lower to the upper zones. The lowest quantities of residue were found in the upper zones. Residue quantities in the

upper external, middle, and central zones were  $1.163 \mu\text{g}/\text{cm}^2$ ,  $0.849 \mu\text{g}/\text{cm}^2$  and  $0.578 \mu\text{g}/\text{cm}^2$ , respectively.

Because the trees that had more leaves on them during the time/season in which droplets of smaller diameters were sprayed by using the knapsack sprayer equipped with an air-assisted rotary disc nozzle, the highest quantities of residue were observed in the lower zones of hazelnut trees.

Hazelnut trees were divided horizontally into external, middle and central zones (from the exterior to the center). The amount of residue was determined for each one of these different zones. The highest quantity of residue was found in the middle external zone with a value of  $1.427 \mu\text{g}/\text{cm}^2$ , while the lowest quantity was found in the upper central zone with a value of  $0.578 \mu\text{g}/\text{cm}^2$ . Penetration from the exterior to the center in the lower and middle zones of hazelnut trees was higher in comparison to the upper zone. This was due to the higher amount of dye residue that was sprayed on the lower and middle zones. In the middle area, the amount of residue on the external, middle and central zones was  $1.427 \mu\text{g}/\text{cm}^2$ ,  $1.003 \mu\text{g}/\text{cm}^2$  and  $0.666 \mu\text{g}/\text{cm}^2$ , respectively. In the upper area, the amount of residue on the external, middle and central zones was  $1.163 \mu\text{g}/\text{cm}^2$ ,  $0.849 \mu\text{g}/\text{cm}^2$  and  $0.578 \mu\text{g}/\text{cm}^2$ , respectively.

As droplets of insecticide settle on the first target surface they touch when being sprayed the number of droplets gradually decreased towards the center of the hazelnut trees. For this reason, the amount of residue that accumulated in the center was also lower according to the other surfaces of tree. Cross et al. (2001b) found that zones of hazelnut trees which were closer to the pulverizer during insecticide application had higher levels of residue than zones that were more distant. Results of the study Balsari et al. (2002) showed that the amount of residue which accumulated in leaves of the outer tree canopy was higher than the amount which accumulated in leaves of the inner zones.

During insecticide applications performed by using knapsack sprayers with air-assisted rotary disc nozzles, the average amount of residue loss was  $0.146 \mu\text{g}/\text{cm}^2$ . The loss observed on surfaces closer to the hazelnut trees was greater than the loss observed on surfaces in more distant locations.

Filter papers indicated that the amount of loss to the air was  $0.22 \mu\text{g}/\text{cm}^2$ . Low volume applications may lead to greater drift losses (Salyani and Cromwell, 1992).

Following the application of the insecticide, a net cage was placed on each hazelnut-yielding branch, and 10 mature nut weevils were placed within each one of these net cages (Figure 3). One cage was formed for each one of the three different doses. Counts were then performed on the first, third, and seventh days following insecticide application (Anonymous, 1996). During these counts, the number of dead, living, and paralyzed mature nut weevils was determined.



Figure 3. Cage studies for assessing biological effectiveness, and the counting of mature nut weevils that was performed after insecticide application.

Statistically significant difference was found between doses that were used in the studies ( $P < 0.01$ ) (Table 1). It was determined that the applied doses had an effect on the results of the cage studies.

Table 1. Biological effectiveness values calculated according to the combined Day 7 counting results of the cage studies

Sprayer type	Doses	Analysis results	Biological effectiveness %
Knapsack sprayer with air-assisted rotary disk nozzle	1/1 dose	9.91	97.34
	3/4 dose	9.70	93.35
	1/2 dose	9.56	90.67
Mean		9.73	
		9.77	
<b>Doses</b>			
1/1 dose		9.96 A	
3/4 dose		9.77 B	
1/2 dose		9.54 C	
<b>Doses</b>		**	
Sprayer		N.S.	
Year		N.S.	
Dose x Sprayer		N.S.	
Year x Dose		N.S.	
Year x Sprayer		N.S.	
Year x Dose x Sprayer		N.S.	
CV		4.32	

\*\* Significant ( $P < 0.01$ ). There are no differences between values indicated with the same letters.  
N.S. Difference between the relevant items was not statistically significant

The biological effectiveness values obtained by combining results of parcel studies performed over two years are presented in Table 2. It can be seen that a statistically significant difference was found between the doses used in the study ( $P < 0.01$ ).

According to the two-year plot study results, 1/1dose applications were found as statistically very significant. The biological effectiveness was determined at 1/1dose applications.

Table 2. Biological effectiveness values calculated according to the combined counting results of the parcel studies

Sprayer type	Doses	Analysis results	Biological effectiveness %
Knapsack sprayer with air-assisted rotary disk nozzle	1/1 dose	9.91	97.25
	¾ dose	9.60	91.03
	½ dose	9.01	80.27
<b>Mean</b>		<b>9.50 B</b>	
<b>Doses</b>			
Full dose		9.97 A	
¾ dose		9.68 B	
½ dose		9.20 C	
<b>Doses</b>		**	
Sprayer		N.S.	
Year		N.S.	
Dose x Sprayer		N.S.	
Year x Dose		N.S.	
Year x Sprayer		N.S.	
Year x Dose X Sprayer		N.S.	
VK		3.69	

### Conclusions

In the studies performed by using the knapsack sprayer with an air-assisted rotary disc nozzle, the  $D_v(50)$  value was measured as  $64.68 \mu\text{m}$  under study conditions.

Regarding the distribution of residue quantities from the upper to the lower zones of hazelnut trees, the highest quantity of residue was found in the middle external zone of the tree with a value of  $1.427 \mu\text{g}/\text{cm}^2$ , while the lowest quantity was found in the upper central zone with a value of  $0.578 \mu\text{g}/\text{cm}^2$ .

The amount of loss to the soil was  $0.146 \mu\text{g}/\text{cm}^2$ , while the amount lost to drift was  $0.22 \mu\text{g}/\text{cm}^2$ .

In the parcel studies for biological effectiveness, the effectiveness value was determined as 97.25% for full dose applications, 91.03% for ¾ dose applications, and 80.27% for ½ dose applications. During the parcel studies, the ½ dose application provided the lowest biological effectiveness value.

In the cage and parcel zones, an average biological effectiveness of 97.3% was obtained. Based on these values, it was determined that standard 20 l/da applications for chemical control can be performed with the sprayer used within the context of this study.

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**FIVE YEARS AFTER THE FIRST RECORD OF *TUTA ABSOLUTA* (MEYRICK) IN ALGERIA, WHAT DO WE EXPECT FROM ITS NATIVE NATURAL ENEMIES?**

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**Abstract**

Since its first record in the vicinity of Mostaganem (Northwestern Algeria) in spring 2008, the tomato leaf miner *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) has established as a key pest of tomato crops. It is the most important pest devastating tomato crop throughout the year. The development of approaches to manage *T. absoluta* in Mediterranean countries is depending of several factors. Many works were initiated on its control and much still remains to be done.

With the aim of gathering more information about practices, we monitored *T. absoluta* infestation in greenhouses. The list of its native enemies is in expansion, reaching nowadays over 10 native species. The mainly species of predators belong to the Miridae family (*Macrolophus pygmaeus*, *Nesidiocoris tenuis* and *Dyciphus tamanii*) and the most important parasitoid species belong to the Eulophidae family with three dominant species ( *Necremnus arthynes* , *Stenomesus* sp and *Neochrysocharis formosa*). The first parasitoid species is found through the country while the second was more recorded in the South part of Algeria. When farmers adopt spontaneously chemical control because not having enough understanding about side effects of pesticides on beneficial organisms there are difficulties in doing available work on biological control. On the other hand unfortunately some native enemies are recorded under a misidentification. Indirect interactions were found have been neglected to explain the parasitism rate.

We are trying to explain what we have to do encouraging acceptable results in greenhouses and open fields. This way seems to us to be necessary because we are in constant suspense between enthusiasm and discouragement.

**Keywords:** *Tuta absoluta*, biological control, native natural enemies, integrated pest management

**Introduction**

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lep: Gelechiidae) is known to be native from South America where it remained confined until 2007. (EPPO, 2007; Desneux et al., 2010). Its initial detection in the Mediterranean basin occurred in Spain in winter 2006/2007 (Urbaneja et al., 2007) likely following the importation of tomatoes from Argentina (Garcia Mari F., personal com.) at the end of 2006. The pest has expanded quickly over all the tomato cropping causing serious damage (Urbaneja et al., 2008). Since it was reported in many Mediterranean countries of Europe, North Africa and Middle East (Desneux et al. 2010; Garcia Mari- et Vercher, 2010). Despite the presence of a well-organized plant protection throughout Europe, no measure has been capable to halt the spread of *T. absoluta* throughout the continent (Desneux et al., 2010).

In Algeria the first sightings of *T. absoluta* occurred in spring 2008 in a tomato greenhouse located in the vicinity of Mostaganem (Northwestern Algeria) (Guenaoui, 2008). In July 2008 the pest was recorded under official control (INPV, 2008; EPPO, 2008). The initial invasion of the pest happened suddenly, requiring a rapid response. The success of its spread in term of distribution and abundance is related to the favourable climatic conditions, wide distribution of its host plants (Solanaceous family) and to a low efficacy of native natural enemies in the new territory because tomato growers applied large quantities of insecticides which affected parasitoid abundance in the vegetable agro-ecosystems and probably promoted the development of pesticide resistance within

the pest. The development of approaches to manage the pest would be facilitated through more knowledge including the data that may help the implementation of efficient biological control programs (Desneux et al. 2010).

The tomato production system in Algerian greenhouses differs from that in Europe. Tomatoes for the fresh market are mainly produced in plastic greenhouses without heating. The economic damage level of tomato fruits is very high, because bad measures are applied against the pest that could lead to more difficulties in pest management in the future. Damage to tomato crops will be limited since the pest is controlled by several methods in greenhouses or outdoors in a strategy IPM but before an effective IPM system could be developed for *T. absoluta* it is necessary to identify the key native natural enemies to know the species composition and abundance predators and parasitoids that will be recognized for their potential contribution to the integrated pest management of *T. absoluta*.

The aim of this work is to give a picture of the composition of the species that spontaneously provide biological control services in Algeria. The first step was to identify native predators and parasitoids, their hosts (leaf miners) and plant hosts. The research project focused on the occurrence of indigenous natural enemies in two tomato growing areas at Mostaganem (Northwestern) and at Touggourt-Biskra- (Southwestern) where the climate is diverse with dry periods and temperatures higher than in the North. The work began in 2009.

## Materials and Methods

### Parasitoids

During cropping seasons (from 2009 to 2012) samples tomato leaves infested by *T. absoluta* were randomly collected from two sites in Mostaganem ( greenhouse and open field) with 250 leaves/week and 2 other sites (100 leaves each) in the South. The samples were placed in plastic bags labeled with the name of the crop, date, location. Infested leaves were examined under a stereomicroscope and larval mines were broken open and all larvae were examined for parasitism. Larvae were recorded as parasitized and kept in vials in the laboratory conditions until emergence of parasitoids. The parasitoids were put in vials with alcohol until identification.

### Predators

Tomato plants were observed in several sites on untreated crops for detecting predators preying on *Tuta absoluta*. The predators were taken with a small aspirator, put in plastic vials labeled with the name of the crop, date, location and brought to the laboratory where individuals were frozen and kept in alcohol until identification.

## Results and discussion

### Parasitoids

Only six species of parasitoids (all Hymenoptera) belonging to the Eulophidae family were identified during the investigated period: *Necremnus artynes*, *Neochrysocharis formosa*, *Stenomesus* sp, *Hemiptarsenus zilahisebessi*, *Diglyphus isaea*, *Pnigalio* sp.

*N. artynes* is the most frequent species in Algeria. It was also reported by Boualem et al. (2012), Kolaï et al. 2012 in the vicinity of Mostaganem and by Dahliz in the Southeastern (unpublished data). The parasitoid has been reported over all Mediterranean countries, in Spain (Gabarra et al., 2010), in France (Desneux et al. 2012) and in Italy (Zappala et al., 2011).

*N. artynes* paralyze the larva before laying the egg adjacent to it. A study on its potential to reduce population of *T. absoluta* in Algerian conditions is achieving.

*Neochrysocharis formosa* was lowly recorded in both sites since 2010. It is likely distributed in almost all of the world. It is a very common parasitoid, recorded from more than 100 host species in many orders (Diptera, Lepidoptera, Coleoptera, Hemiptera.) (Noyes, 2013). It was also reported as an hyperparasitoid of the primary parasitoid *Diglyphus isaea* (Hym: Eulophidae) (Akihito, 2002). In

Japan an asexual strain of *Neochrysocharis formosa* was registered as a biological control agent for agromyzid leaf miner pests. The native strain recorded on *T. absoluta* is arrhenotokous.

*Stenomesus* sp was recorded only in the Southeastern. It is adapted to high temperatures ( Dahliz and Guenaoui , unpublished data). *Stenomesus* species and mainly *S.japonicus* (Ashmed) have a wide host range, recorded from Gelechiidae, Pyralidae, and Gracillariidae (David and Stevens, 1992).

*H. zilahisebessi*, *D. isaea* (1 specimen) and *Pnigalio* (2 specimens) were recorded on *T. absoluta* only at Mostaganem. *H. zilahisebessi* was recorded in Spain (Gabarra and Arno, 2010).

*D. isaea* could not be candidate because the parasitism failed on *T. absoluta*.

The rate of each species varied according to the site, the season and the year .

The larval ectoparasitoid *Dineulophus phthorimaeae* (Hym: Eulophidae) that is described as native to Argentine and Chile by Savino et al. (2012) was reported in Algeria by Benmoussa et al. (2009) but we never found it in our samples.

All species recorded are idiobiont species that halt host development after attacking it by the injection of venom. They feed on their host and prefer the third instar larval host to oviposit , grow protected inside the leaf mines and pupate *in situ* (Dahliz et Guenaoui unpublished data).

### **Predators**

In the Northwestern until June 2012, only five species of predators had been seen preying on *T. absoluta* .There are three Miridae, one Formicidae and recently one Chrysopidae. The Miridae *Macrolophus pygmaeus* (Rambur) *Nesidiocoris tenuis* (Reuter) and *Dicyphus tamaninii* ( Wagner) have been observed to prey on *T. absoluta* in the vicinity of Mostaganem. *M. pygmaeus* is sometimes confused with *M. caliginosus* (synonym of *M. melanotoma*) ( Martinez-Cascales et al. 2006 ).The most important of them is *N. tenuis* more active and tolerant to high temperatures. During 2011, *Tapinoma nigerrimu* (Hym:Formicidae) a common ant in Algeria,was seen preying on infested tomato fruits playing a major rôle. Recently larvae of *Chrysopa carnea* (Neuroptera: Chrysopidae) were found preying actively on larvae of *T. absoluta* on the hedge of the tomato greenhouse (Pers. Observation).

As a first step for evaluating the efficacy of the native predatory mirids on *T. absoluta* laboratory experiments were conducted in 2010/2011 at 25°C.The results showed that both bugs preyed actively on eggs and first larval instars (Guenaoui et al. 2010; Guenaoui et al., 2011). In Spain, Urbaneja et al. (2008) showed the prey suitability of the tomato borer *Tuta absoluta* for the Spanish strain of *M. pygmaeus* and *N. tenuis*. The last species is a commercial product known under NESIBUG name and sealed by Koppert Biological System.This commercial strain has been first imported in 2010 by the Algerian Ministry of Agriculture for releasing in tomato greenhouses throughout different sites . In 2013 several releases occurred (INPV, 2013) In Algeria, in despite of a large use of chemical control little is known on the evaluation of effects of pesticides on released predators .In Spain several studies had been conducted to test the toxicity of different products used in greenhouses (Arno and Gabbara, 2011). More prospects are need in Mediterranean countries for biological control of *T. absoluta* (Urbaneja et al., 2012).

### **Conclusion**

In conclusion, this work provides some data on natural enemies associated with *T. absoluta* in fields during the past five years. The spread of this invasive pest may provide an opportunity to learn more about the pest (a possible parthenogenetic reproduction) and its natural enemies. Concerning predatory bugs the importation of *N. tenuis* for releasing in specific sites to encourage the use of biological control, we have a critical regard because this could lead to interactions of competition with the native strains for the same resource. The releases will be too expensive for the tomato grower when he should pay himself the treatment at cost price. In this case, biological control that is the main tactic inducing a reduction of insecticides use will be neglected. We have to learn to



vegetable growers they should preserve safety of the beneficial with selective pesticides. The efficacy of Emamectine against larvae of *T. absoluta* was tested (Gasmi and Guenaoui, 2012) Its toxicity towards natural enemies studies have been shown only towards Coccinellidae (Youn et al. 2003).

The most abundant parasitoid is *Necremnus artynes* which has a large distribution over the Mediterranean basin. In Algeria its ability to affect strongly the populations of *T. absoluta* is not yet proved because we expect that the parasitism rate will be very low when pest populations are high. Native entomophagous need time to be adapted to *T. absoluta* and to control it effectively by adjusting their behavior and physiology to the new pest. So, it is essential to gain more knowledge to understand the relations between host plants, the pest and its natural enemies for a real national program of biological control. For this, we need well-trained agents and growers who can help researchers to obtain the biologic information needed to evaluate different approaches and separate effective methods from ineffective ones.

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## AN EXAMPLE OF CROATIAN LABELING FOR PROTECTION OF AGRICULTURAL FOOD PRODUCTS

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### Abstract

Food markets are saturated with highly competitive products that often do not possess the adequate quality. For this reason, consumers are uncertain about the quality and origin of the food products that they intend to buy. European Commission (EC) recognizes this problem and in 1992 created labels for protection of agricultural food products, known as “Protected Designation of Origin” (PDO), “Protected Geographical Indications” (PGI) and “Traditional Specialty Guarantee” (TSG). Croatia adopted these labels in 1995, since then, 4 products are registered with PDO label, 8 products with PGI label and one product is under the process of getting PDO label. At the beginning of 2012 the project aimed to protect the geographical origin of “Vrgorac strawberries” was initiated by strawberry producers’ association. The aim of the project is to enhance production, increase competitiveness and quality of strawberries from the area of Vrgorac. In the survey the entire producing area of “Vrgorac strawberry” was involved, including the fields Rastok, Kotezi, Kokorici, Prapatnica, Jezero and Pojezerje. Furthermore, in this project, supported by the Ministry of Agriculture of Croatia, the production processes and the production range were standardized, and the most important chemical, morphological and organoleptic characteristics of the strawberries from that area were described. Based on the obtained results, a specification of “Vrgorac strawberry” will be drafted and submitted to the procedure for getting the protection of geographical indications label.

**Keywords:** labels, protection of food products, protected geographical indications, evaluation, strawberry

### Introduction

Food markets worldwide are saturated with highly competitive products that often do not possess the adequate quality. In those competitive markets, consumers have begun to increasingly recognize and appreciate products that are characterized by a special quality. Those high quality products have to be protected and labelled by labels; “Protected Designation of Origin” (PDO), “Protected Geographical Indications” (PGI) and “Traditional Specialty Guarantee” (TSG), to avoid adulteration and misleading of consumers. For this reason, in 1992 European Commission (EC) created these labels to protect agricultural food products and also to protect consumers. Those labels were defined in European Union (EU) by Council Regulation (EEC) 2081/92. In 2006 this Regulation was replaced by a new EU Council Regulation (EEC) 510/06 on the protection of geographical indications and designations of origin for agricultural and food products. Croatia adopted all EU Regulations on labelling of food products. At the beginning it was in the responsibility of State Intellectual Property Office, and in 2003 it is transferred to the Ministry of Agriculture. The Ministry of Agriculture has issued an Act on PDO, PGI and TSG of agricultural food products (NN 50/12), which, among other things, Croatian legislation taken from Council Regulation 510/06 on the PDO, PGI and TSG. In 2012, Croatian Ministry of Agriculture complemented the old Regulation (NN. 50/12) with a new one NN 102/12 which is complied with EEC 1898/96.

Vrgorac strawberry is characterized by high quality product and became a successful brand on markets in Croatia and abroad. Resellers recognized this brand and usually label the strawberries from other breeding areas or even from import as “Vrgoracka strawberry” to achieve a higher market price. To protect strawberry producers from Vrgorac area and consumers “Association of Vrgorac strawberry” started the project to label the Vrgorac strawberry with PGI.

PGI is defined as name of a region, a specific place, or in exceptional cases, a country that is used to describe an agricultural food products; if product originate from that region, specific place or country; if possesses a specific quality, reputation or other characteristics to its geographical origin; or if the production and / or processing and / or preparation takes place in that geographical area.

### **Materials and methods**

This study was done within the project “Vrgorac strawberry-local product for the global market” which is financed by the Croatian Ministry of Agriculture. In accordance with project design, authors made a project protocol, which consists of several separate ensembles. The first part of the project was the selection of locations for sampling strawberries, soil and water. After that carrying out a survey on the cultivation of strawberries in Vrgorac area, strawberry sampling, sample analysis and analysis of the results. The last step of the project protocol was preparation of the food product specification, in accordance with all relevant regulations for labeling Vrgorac strawberry with PGI (NN 102/12, EEC 1898/96).

### **Results and discussion**

The process of obtaining the PGI in Croatia is defined in the Regulation NN. 102/12 and right to apply for PGI has any association of producers and / or processors (regardless of their legal status, which the association has) and individual manufacturer and / or processor (either natural or legal persons). The procedure for registration of food product with PGI is under the responsibility of Ministry of Agriculture in Sector for labeling and food quality in the Department of indigenous products. An application for the registration of food product with PGI shall contain: the registration form tag; form for registration of users of registered mark; details about applicant; label that intends to register or use; product specification; the name of the certification body and the written consent of that body; certificate of conformity and summary of the application.

Product specification is the most important document for the registration of food products with PDO, PGI and TSG labels. Even that specification is the part of the application for PGI it is important because it represents the essence of the label protection of the product and must be developed in detail. Producers are obliged to its responsibility to make product specification. According to the specification, the certification body makes control plan (approved by the Ministry of Agriculture) to issue certificates of conformity. Minister authorizes legal entities (certification authority) which is supervised by the Ministry and Croatian Accreditation Agency.

Content of the product specification:

- Product name
- Description of product
- Geographical area
- Proof of origin
- Description of a method of obtaining a product
- The connection between the product and geographical area
  - a) details of the geographical area
  - b) details of the product quality
  - c) a causal link between geographical area and product

- Information about certification body
- Specific labeling rules
- Requirements prescribed by special regulations
- Historical evidence

The right to use the registered PGI belongs to all the producers and/or processors of Vrgorac strawberry that are the members of “Association of Vrgorac strawberry” and as long as they meet the requirements. From 2006 Croatian producers can apply for registration of a designation of origin and geographical indications directly to the EC ( Regulation No. 510/2006). Application forms are available on the website of the European Commission (<http://europa.eu.int/comm/agriculture>).

After collecting all necessary data, historical evidences, chemical and morphological analysis of Vrgorac strawberry, authors made product specification. Vrgorac strawberry and area of Vrgorac is described in detail and quality that this strawberry possesses is connected to the geographical area. Strawberries from Vrgorac with all their specific characteristics belongs to the class of premium product that is possible to grow with that characteristics only in area of Vrgorac.

### Conclusion

Getting the PGI label for Vrgorac strawberry will protect the producers from this area from adulteration and protect misleading of consumers. This label will also have an important socio-economic aspect on development of this rural area. From the economic point of view; higher market price of the product; recognition of region and product; ability to export and development of tourism. From the point of view of Legal aspect, PGI label will contribute in protection from abuse and the reputation of the original product. Consumers will increase demand for this local product and labels guarantee recognizable quality. From the Social aspect point of view, PGI label will prevent the outflow of population from the region (keep the rural citizens in the rural area) and increase producers income.

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## OCHRATOXIN A AND OCHRATOXIGENIC FUNGI IN TUNISIAN GRAPES AND WINE

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### Abstract

This work summarizes the results of a large study on the occurrence of ochratoxigenic fungi and Ochratoxin A (= OTA) from wine and table grapes in Tunisia.

Black aspergilli were the dominant genus among the filamentous fungi isolated from grapes and were the only potential OTA-producing fungi found. The most abundant species were member of *Aspergillus niger* aggregate than *Aspergillus carbonarius*. Uniseriate aspergilli were rarely present. Of the *A. carbonarius* isolates, 97% were OTA positive but only 3% of the *A. niger* aggregate isolates produce this toxin. During grape maturation, the frequency of black aspergilli increased due to increase of the number of *A. carbonarius*.

Thereafter musts produced from mature grapes were analysed for their OTA content. More than the half of the samples contained detectable levels of OTA, (between 0.01 and 5.85 µg OTA l<sup>-1</sup>). The most contaminated musts were obtained from the region of Raf-Raf located in the North-Est and characterized by a humid climate, however, musts obtained from the region of Regueb located in the center, which is a new area for the grapevine cultivation and characterized with an arid climate were rarely contaminated.

For the contamination of tunisian wine, OTA was detected 85% of the analyzed samples. The results show OTA levels ranged between 0.09 and 1.5 µg/L. Neither of the studied samples shown levels above the European regulatory limit (2 µg/L).

**Keywords:** Black aspergilli, grape, Ochratoxin A; Tunisian wine

### Introduction

Ochratoxin A (OTA) is a toxic secondary metabolite produced by moulds belonging to several species of the genera *Aspergillus* and *Penicillium*. It is a potent nephrotoxin and hepatotoxin with teratogenic, mutagenic and immunosuppressive effects (Pitt et al., 2001). Ochratoxin A was first detected in wine by Zimmerli and Dick (1996). Since then, presence of OTA in grape and its derived products, such as dried vine fruits, grape juices and wine has been worldwide reported (Varga et al. 2007). The European Union legislation authorities have introduced an OTA limit of 2 µg/L in wine, must or grape juice (European Commission, 2005). Ochratoxin A is produced by species in *Aspergillus* sections *Nigri* (black aspergilli) and *Circumdati*, commonly found in warm and tropical climates. *Penicillium verrucosum* is considered as the main source in temperate climates and is more frequently associated with cereals (Pitt and Hocking, 1997). These authors found that among black aspergilli, *A. carbonarius* was the main species producing OTA in grapes. Some members of the *A. niger* aggregate have also been found to produce OTA. It was observed that wines from Southern Europe and North Africa, with Mediterranean climates, contained more OTA than those originating from the more temperate regions of central Europe (Zimmerli and Dick, 1996; Ottener and Majerus, 2000). In fact, it was found that climate has an effect on the occurrence of OTA and OTA-producing fungi in grapes (Battilani et al., 2006a, 2006b; Serra et al., 2006). After cereals, the wine is the second food with the greatest contribution in the daily intake of OTA by the population of European Union. In order to reduce consumers' exposure, EFSA recommended maximum tolerable daily intakes of 120 ng/kg.bw/week. Wine production in Tunisia

is estimated to 300.000 Hectoliters per year. The annual exportation overtakes 140.000 Hectoliters, essentially, to European countries. Beer production in Tunisia is estimated to 1.000.000 Hectoliter intended exclusively to local consumption. The aim of this work was to survey the contamination of Tunisian grapes and wines with OTA

### Materials and methods

#### Sampling

The survey was carried out over 3 years and involved four vineyards located in four viticultural regions of Tunisia (Belli: Grombalia region, Baddar: Cap bon region, Regueb: Center of Tunisia, Raf-Raf: North east of Tunisia) with specific climate. Six grape varieties in each vineyard, representative of their grape-growing area were selected for further study. The varieties included wine and table grapes. Grape samples were harvested at maturity stage (End of July/August) depending on the region and variety. Ten plants along diagonal transects were chosen in each vineyard and one bunch of grapes was taken from each plant. Bunches were kept in sterile bags and transported in cooled boxes (4 °C) to the laboratory for analysis. A total of 34 wine samples (18 red, 9 rosé and 7 white) produced in Tunisia were also purchased, in commercially available size, from different supermarkets in Tunis (Tunisia). The wine samples were produced between 2004 and 2006 and consisted of various varieties from different vintages (designation of origin). The samples were stored in their original containers in fridge at 4°C until OTA analysis.

#### Isolation and identification of fungal flora

Berries were surface-disinfected with hypochlorite (15%) Afterwards, they were plated in Petri dishes containing Malt Extract Agar medium (MEA) with Chloramphenicol (100 mg.l<sup>-1</sup>). Plates were incubated at 25°C for 10 days. All the colonies of potential OTA- producing fungi (*Aspergillus* and *Penicillium*) were classified into genera. For species identification, the *Aspergillus* isolates were cultivated in Czapek Yeast Agar medium (CYA) and incubated at 25°C for 5 days. Black *Aspergillus* species were classified into three groups (uniseriates, *Aspergillus niger* aggregate and *Aspergillus carbonarius*) in accordance with Dr. Kozakiewicz guidelines (CABI Bioscience, Egham, UK).

#### Analysis of OTA in grapes and wines

After selecting berries for fungal isolation, the ten bunches collected from each vineyard and variety were manually crushed and the resulting musts (n=72) were analyzed for OTA content according to the method of Bezzo et al. (2002). Musts (100 ml) were first centrifuged (2500 g, 20 min), liquid phase was, brought to a pH 7.4 (NaOH 4M), filtered and applied to an immunoaffinity column (Ochraprep, Rhône Diagnostics Technologies Ltd, Glasgow, UK). For wine samples no centrifugation was realized. The column was then washed with 20 ml of distilled water and dried up with an air stream. Elution was realized with 1.5 ml of methanol/acid acetic (98/2) solution. The eluate was evaporated to dryness, dissolved with 0.8 ml of mobile phase and injected into the HPLC system equipped with a fluorescence detector (  $\lambda_{exc}$  230 nm;  $\lambda_{em}$  458 nm) and a reversed-phase column C18 (Waters Spherisorb 5 µm, ODS 2, 4.6 × 250 mm, Milford, MA, USA). The mobile phase was composed by a sodium acetate 4 mM/acetic acid (19:1: v/v) solution (52%) and acetonitrile (48%). Detection and quantification limits (LOD and LOQ) were 0.03 µg OTA/l and 0.05 µg OTA/L, respectively. The recovery for OTA on grape juice samples was 95 ± 4% (mean ± SD., n = 3).

### Results and discussion

#### Distribution of black *Aspergillus* species

A total of 1242 black aspergilli were isolated along the three sampling three years distributed in the four regions from Tunisia (Belli, Baddar, Raf-Raf and Regueb). The analysis of variance revealed

that the single factor region and the interaction year and region presented significant differences in the number of black aspergilli isolated ( $P < 0.01$ ). The sampling year does not affect the fungal colonisation grapes ( $P > 0.05$ ). Grapes from Regueb were significantly the most contaminated with black aspergilli each year. No statistical differences were found between the number of these moulds in the regions of Baddar, Belli and RafeRaf ( $P \geq 0.05$ ). The distribution of black aspergilli isolates is presented in Table 1.

Table1: Percentage of black aspergilli isolates. Numbers in brackets are the number of black aspergilli isolates of each group isolated/the total number of black aspergilli.

Group		Region			
		Baddar	Belli	Regueb	Rafraf
Uniseriate	1 <sup>st</sup> Year	2.9 (4/136)	1.0 (1/101)	1.9 (2/104)	0 (0/101)
	2 <sup>nd</sup> Year	4.8 (4/84)	1.1 (1/94)	10.0 (13/130)	0 (0/115)
	3rd Year	0 (0/77)	1.6 (1/63)	0.7 (1/150)	2.3 (2/87)
	Total	2.5 (8/297)	1.2 (3/258)	4.2 (16/384)	0.7 (2/303)
<i>A. carbonarius</i>	1 <sup>st</sup> Year	19.9 (27/136)	24.8 (25/101)	2.9 (3/104)	20.8 (21/101)
	2 <sup>nd</sup> Year	23.8 (20/84)	41.5 (39/94)	0 (0/130)	22.6 (26/115)
	3rd Year	58.4 (45/77)	74.6 (47/63)	0.7 (1/150)	18.4 (16/87)
	Total	30.9 (92/297)	43.0 (111/258)	1.0 (4/384)	20.8 (63/303)
<i>A. niger aggregate</i>	1 <sup>st</sup> Year	76.5 (104/136)	76.2 (77/101)	94.2 (98/104)	79.2 (80/101)
	2 <sup>nd</sup> Year	60.7 (51/84)	57.4 (54/94)	90 (117/130)	77.4 (89/115)
	3rd Year	98.7 (148/150)	30.2 (19/63)	36.4 (28/77)	79.3 (69/87)
	Total	61.6 (183/297)	58.1 (150/258)	94.5 (363/384)	78.5 (238/303)

Isolates of *A. niger aggregate* formed the dominant group in the four regions, especially in the region of Regueb where they represent 94.5% of the isolated black aspergilli. In contrast, uniseriate isolates were recovered in small numbers in the different regions. At each sampling year, the highest level of *A. carbonarius* infection was observed in the region of RafeRaf where it represented a mean of 43% of black aspergilli isolated. The level of contamination by this specie was lower in the regions of Baddar and Belli with, respectively, 30.9% and 20.8% of black aspergilli. The incidence of *A. carbonarius* was very low in Regueb region where only four *A. carbonarius* (1% of black aspergilli) were isolated during the three sampling years.

### OTA contamination of grapes

The data related to the OTA concentrations in musts are presented in table Table 1.

The results showed that at maturity stage, 54% (39/72) of analyzed samples were contaminated with 10% (4/39) of OTA positive samples exceeding the EU restrictive limit ( $> 2 \mu\text{g/L}$ ). These samples came from Raf-Raf (5.44 and 5.84  $\mu\text{g OTA/L}$ ) and Baddar (3.27 and 2.23  $\mu\text{g OTA/L}$ ) regions. Eighty three percent (15/18) of grapes from Raf-Raf were OTA contaminated in the range of 0.06-5.85  $\mu\text{g/L}$ . In Baddar, 83% (15/18) of grape samples contained between 0.06 and 3.27  $\mu\text{g OTA/L}$ . Forty four percent (8/18) of grapes from Belli contained between 0.09 and 0.96  $\mu\text{g OTA/L}$ . Among the grape samples originating from Regueb, only one sample was contaminated with OTA at a very low concentration (0.05  $\mu\text{g/L}$ ). The multiple comparison test ( $P=0.05$ ) showed that the OTA contamination of grape is significantly different between Regueb region and the regions of Baddar and Raf-Raf. A significant difference for OTA contamination was also found between Belli and Raf-Raf regions. The distribution of the OTA concentrations in the grapes showed that higher OTA concentrations were detected in grapes from Raf-Raf with 40% of samples containing between 0.5 and 2  $\mu\text{g OTA/L}$  and 13% containing more than 2  $\mu\text{g OTA/L}$ . For grapes originating from Baddar,



20% of samples were contaminated in the range of 0.5-2  $\mu\text{g}$  OTA/L and 13% of samples exceeded 2  $\mu\text{g}$  OTA /L. This level of contamination is higher than observed in Belli were 75% of samples contained less than 0.5  $\mu\text{g}$  OTA/L and 25% were contaminated with concentrations ranging between 0.5 and 1  $\mu\text{g}$  OTA/L.

Table 2: OTA concentrations in grapes collected from the four regions in the different years ( $\mu\text{g}/\text{L}$ ).

Region	Variety	Sampling year		
		1 <sup>st</sup> year	2 <sup>nd</sup> year	3 <sup>rd</sup> year
<b>Baddar</b> (Est part of Tunisia Cap bon region)	Muscat Alexandrie	0.97	3.27	0.25
	Cabernet Sauvignon	0.14	1.53	<LQ*
	Ugni Blanc	0.06	1.21	0.11
	Italia	0.17	0.24	0.38
	Syrah	0.38	0.12	<LQ
	Carignan	<LQ	0.16	2.23
<b>Belli</b> (North part of Tunisia)	Chardonnay	0.18	0.13	0.96
	Muscat Alexandrie	<LQ	0.35	<LQ
	Cabernet Sauvignon	<LQ	0.10	<LQ
	Italia	<LQ	0.77	<LQ
	Syrah	0.25	<LQ	<LQ
	Carignan	0.13	<LQ	<LQ
<b>Raf-Raf</b> (North-Est part of Tunisia)	Rezeki	0.1	1.49	0.96
	Bezoul Khadem	0.12	<LQ	5.85
	Akhal Raf-Raf	<LQ	0.15	0.27
	Marsaoui	0.18	0.55	<LQ
	Bid Hmam	1.88	0.07	0.85
	Muscat Raf-Raf	0.36	5.45	1.09
<b>Regueb</b> (Center part of Tunisia)	Italia	<LQ	<LQ	<LQ
	Victoria	<LQ	0.05	<LQ
	Sultanine	<LQ	<LQ	<LQ
	Superior Seedless 1	<LQ	<LQ	<LQ
	Matilde	<LQ	<LQ	<LQ
	Superior Seedless 2	<LQ	<LQ	<LQ

\*LQ (0.05  $\mu\text{g}/\text{L}$ )

These results showed the presence of the toxin in both wine and table grapes at the three sampling years. Almost half of samples were contaminated with OTA which is similar to the level of contamination detected in Southern Italy (Lucchetta et al., 2010). Among the OTA positive samples, 4 out of 39 exceeded 2  $\mu\text{g}/\text{l}$ . However, it's important to note that grape collected at maturity will undergo several stages (transport, storage, vinification, drying) which may influence

the OTA final concentration of grape product. Several factors affect the grape colonization by ochratoxigenic moulds and the OTA contamination, in particular, geographic and climatic conditions. In several European countries, southern regions located in the Mediterranean basin, were found to be particularly affected (Battilani et al., 2006a; Battilani et al., 2006b). Tunisia is a small country with an extended Mediterranean coast; however, areas of grape planting and production are characterized by different climatic characteristics. In Regueb region characterized by an arid climate vineyards grapes were rarely contaminated with OTA and the level detected was very low. The region of Raf-Raf is a coastal region located in the North-Est of the country. It is characterized by a sub-humid climate. These climatic conditions seem to be very favorable to OTA accumulation Baddar and Belli are located in Cap-Bon region (North) which is the main viticulture area in Tunisia and especially for wine grape. This area has a typically semi-arid Mediterranean climate.

For OTA contamination, no difference was found between the two regions, thus the incidence of OTA in grapes depend not only on climatic conditions but also on other factors related to the variety and crop management, such as training system, irrigation and phytosanitary treatments, which influence on the ecosystem of the vine.

### 3.2. Occurrence of OTA in wine

In the present study, a total of 34 wine samples from designation of origin from Tunisia were analyzed and the results are shown in Table 3.

Table 3: Occurrence and OTA levels in wines produced in Tunisia.

Wine sample	Vintage	No. of samples	No. of samples with OTA level > LQ* ( $\mu\text{g/L}$ )
Red	1 <sup>st</sup> year	8	8 (0.5, 0.51, 0.94, 0.44, 0.28, 0.3, 0.38, 0.55)
	2 <sup>nd</sup> year	2	1 (0.53)
	3 <sup>rd</sup> year	8	5 (0.18, 0.43, 0.29, 0.19, 0.09)
Rosé	1 <sup>st</sup> year	1	1 (0.64)
	2 <sup>nd</sup> year	1	1 (0.12)
	3 <sup>rd</sup> year	7	6 (0.22, 0.20, 0.09, 0.15, 0.22, 0.25)
White	1 <sup>st</sup> year	1	1 (0.48)
	2 <sup>nd</sup> year	2	2 (0.92, 1.50)
	3 <sup>rd</sup> year	4	3 (0.39, 0.12, 0.11)

\*LQ (0.05  $\mu\text{g/L}$ )

Eighty-five percent (85%) of the samples contained detectable amounts of OTA ranged from 0.09 to 1.50  $\mu\text{g/L}$  and a mean value of  $0.38 \pm 0.31$   $\mu\text{g/L}$ . The European Union has proposed 2  $\mu\text{g/L}$  of OTA in wine as a maximum residue level. Neither of the studied samples shown levels above the European regulatory limit. The incidence of OTA-positive samples for red, rosé and white sample was 78%, 100% and 86%, respectively. Mean OTA levels and range of contamination in red, rosé and white wines were 0.40 (0.09–0.94  $\mu\text{g/L}$ ), 0.22 (0.09–0.64  $\mu\text{g/L}$ ) and 0.59  $\mu\text{g/L}$  (0.11–1.5  $\mu\text{g/L}$ ), respectively. Different studies in Europe have shown that the wines from the Southern regions usually contain higher OTA concentrations than from the North due to the climate being characterized by high humidity and high temperature (Battilani et al., 2006a). The wines produced in Tunisia come mostly from vineyards located in the North-Est of the country, which is characterized by Mediterranean weather with a semi-arid to semi-humid climate. In several studies conducted in different European countries, red wines were usually found more contaminated with OTA than rosé and white wine (Mateo et al., 2005). Although the number of samples analyzed in our study is low for a relevant discussion about differences in OTA concentrations between the type of wine OTA was found with high concentration in both white and red wines. The higher values of OTA were found in white wines (0.9 and 1.5  $\mu\text{g/L}$ ) and in a red wine (0.94  $\mu\text{g/L}$ ). Several studies reported that dessert wines are quite prone to be contaminated with OTA (Mateo et al., 2007). No dessert wine is produced in Tunisia.

### Conclusion

The occurrence of OTA has been determined in grapes and wines produced in Tunisia. A high frequency of OTA contaminated sample was also found in Tunisian wines; however, none of the analyzed wines contained OTA at levels above the limit fixed by the European Union. Thus, there is no risk for the Tunisian wine exportations. For grape contamination; the results of the present work demonstrated the high risk of OTA in Northern Tunisia, especially for coastal regions.

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**POTENTIAL OF ENTOMOPATHOGENIC NEMATODES APPLICATION AGAINST  
*LIRIOMYZA HUIDOBRENSIS BLANCHARD IN LEBANON***

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**Abstract**

*Liriomyza huidobrensis* Blanchard, the pea leafminer, started to be threaten for vegetable products in Lebanon since the beginning of 1990s. Due to the wide range of insecticide resistance, the control of *L. huidobrensis* by chemicals remains a great challenge and it is important, therefore, to find a friendly environmental control programme against this pest. This study addresses the potential of applying a biological control agent, entomopathogenic nematode against *L. huidobrensis in vitro*. Entomopathogenic nematodes (EPNs) are parasites of soil-dwelling insects that occur in natural and agricultural soils around the world. Thanks to their entomotoxicity, EPNs are good tools for biological control in agriculture almost everywhere in the world.

In the current study, one indigenous strain of EPNs, *Heterorhabditis indica*, was sampled on the coastal area in Lebanon and tested against *L. huidobrensis* pupae *in vitro*. Assays consisted of placing Petri dishes containing sterilized soil and entomopathogenic nematode solution in contact with the pupae of the pea leafminer. While previous studies used larval stages, in the current study, pathogenicity of EPNs is tested *in vitro* against *L. huidobrensis* pupae stage for the first time. Out of 150 pupae used during the experiment, 16 ±1.5 % of the pupae emerged into adults of *L. huidobrensis* and 21±2.5% of the pupae were parasited by another *Liriomyza* natural pathogene - *Diglyphus isaea* Walker. Results showed the mortality of 53±1.5% for the *L. huidobrensis* pupae following the application of entomopathogenic nematodes without any emergence of infestive juveniles nematodes, one month following the infestation. The control tests showed that percentage of emergence from pupae were 79±2%. Comparison with the control tests indicates that 53±1.5% of the *L. huidobrensis* pupae are potentially parasited by *H. indica*. The indigenous strain in Lebanon, *H. indica* can therefore be considered as potential agent in biological control regarding its capability to cause pupae mortality *in vitro* and being isolated in favorable environmental conditions to the presence of *L. huidobrensis* pupae which could prevent field trial failures in further studies.

**Keywords:** *Liriomyza huidobrensis*, biological control, Lebanon, entomopathogenic nematodes, *Heterorhabditis indica*

**Introduction**

*Liriomyza huidobrensis* (Blanchard, 1926), a pea leafminer, originated in South America that has spread to other continents with significance damages to agriculture in recent years (Scheffer et al., 2001). Considered as a highly polyphagous leafminer, it is capable of inflicting severe damage to crop and ornamental host plants including field and glasshouse grown vegetables and flowers. Cited for the first time in California in 1945 as a pest of peas and spinach (Lange, 1945), the invasion of *Liriomyza huidobrensis* started at the end of the 80's in England, France, Belgium and the Netherlands (Sunderland et al., 1992). The species reached the Middle East countries in the beginning of 1990s (Weintraub, 1995). Local farmers in Lebanon noticed first outbreak of leafminers' on gebara plants and the attack of a series of crops included leafy vegetables, many greenhouse and field crops such as cucumber and beans. Within the order of Diptera, *Liriomyza*

*huidobrensis* belongs to the family Agromyzidae. The genus *Liriomyza* was discovered in 1894 with more than 300 species recorded (Parrella, 1987). Within this genus, 23 species are considered economically-important vegetables and ornamental plants in both, glasshouses and outdoors due to their leafmining activity. *Liriomyza huidobrensis* adults are small flies (1.3-2.3 mm length) and their flight range is limited. Under laboratory conditions, a female lays about 100 eggs in total (Hincapié *et al.*, 1993). Eggs are deposited within leaf tissue (CABI, 2004; Weintraub and Horowitz, 1995) between the 4th and 10th day of the adult life (Parrella, 1984). Larvae starts feeding on the spongy mesophyll of the leaf immediately after hatching. Three larval instars develop in the leaf. Full grown larvae make an exit hole in the leaf surface through which it emerges to pupate on the soil surface. There is also a fourth larval stage (prepupae) between the puparium formation and actual pupation (Fig. 1). Pupae varies in colour from light brown to almost black, but all instars are combined and referred to “larvae duration” because of the difficulty in separating larval instars.

The duration of the pupal stage varies with temperature but at least 50% of the total development time of a *L. huidobrensis* individuals is spent in this stage. The life cycle can be completed in 16-43 days at 25 and 15 °C, respectively (Lanzoni *et al.*, 2002). Although *L. huidobrensis* is endemic to warm climates, it has demonstrated the ability to survive cold temperatures and extend its range through supercooling (Chen and Kang, 2004).

Fig. 5. Lifecycle of leafminer (*Liriomyza* spp.) (Enkegaard, 1990)

*Liriomyza* spp. can impact crops in at least six ways: (1) by transmission bacterial and fungal diseases (2) by destroying young seedlings, (3) by causing reduction in field crops, (4) by causing leaf drop above developing fruits (‘‘sunburning’’ of the fruit), (5) by reducing the aesthetic value of ornamental plants and (6) by causing some plant series to be quarantined (Parrella, 1987). The most serious damage is caused by larval feeding. The mining activity of the larvae can reduce the photosynthetic capacity of the plant. Heavy infestation causes desiccation and premature fall of leaves. On the other hand, feeding punctures made by the adult females can also be invaded by fungi and bacteria (Price and Hardbaugh, 1981).

*Liriomyza huidobrensis* is a highly polyphagous species and feeds on a large number of flowers, vegetables and weeds (Weintraub and Horowitz, 1995). It attacks different plant families such as Cucurbitaceae (cucumber, melon), Chenopodiaceae (spinach), Solanaceae (pepper, tomato, eggplant), Violaceae (*Viola* spp.).

Due to the wide range of insecticide resistance, the control of *L. huidobrensis* by chemicals remains a great challenge especially moreover because it is difficult to implement biological control for this pest where it is not indigenous (Jayaraj and Rabindra, 1992). With the increasing awareness of society's concern about pesticide residues in food and the desire for a healthy and aesthetic environment, the use of biorational insecticides for the control of the leafminer is a primary factor in its management.

Entomopathogenic nematodes (EPNs) are known for their potential to attach to the insect cuticle and lodge in their intestine a symbiotic bacteria essential for parasitic success. *Steinernema* and *Heterorhabditis* species are the only insect-parasitic nematodes genera that possess biological control attributes. The complex EPNs-bacteria penetrate through natural insect openings to the hemocele, release the symbiotic bacterial cells that multiply and cause the insect death within 48 h. Since these nematode-bacteria complexes are highly virulent to insects, they are considered as one of the best non-chemical insect pest control alternatives. This characteristic is largely exploited for biological control of insect pests in natural and agricultural soils around the world (Hominick, 2002). Many different insect pests are susceptible to infection by these entomopathogenic nematodes, yet no adverse effects have been shown against beneficial insects or other non targets in field studies (Georgis and Gaugler, 1991; Akhurst and Smith, 2002).

In an attempt to find an environmentally safe control measure against *L. huidobrensis*, in Lebanon the main objective of the present research aimed to make use of one indigenous biological control agent from the entomopathogenic nematodes families for the pest control.

## Materials and methods

### Collection of *Liriomyza huidobrensis*

Potential hosts of *Liriomyza huidobrensis* included cultivated host plants and wild host plants such as *Solanum oleracelus*, *Pisum sativum*. In the present study open fields of *Solanum oleracelus*, main host of *L. huidobrensis* in Lebanon, were chosen along the northern coastal area in Lebanon for collecting *L. huidobrensis* pupae during spring season of 2013 (March-April). Additionally, pupae were collected from agricultural fields of fava beans also. Infested leaves are placed in bags and transferred to the laboratory where *L. huidobrensis* Blanchard pupae are directly isolated from the leaves. The pupae are placed in the refrigerator at 4°C. The pupae are then removed and used in the experiments. Pupae of *L. huidobrensis* can be stored for a maximum two weeks after which the mortality starts to increase.

### Nematodes for pathogenicity experiments

An indigenous entomopathogenic nematode, *Heterorhabditis indica*, isolated from a banana field on the coastal area of Lebanon was used for the experiment. The nematode was reproduced on *Galleria mellonella* larvae at 25 °C in laboratory conditions (Poinar, 1979): ten *Galleria* larvae were placed in Petri dish containing autoclaved soil in contact with a solution of *H. indica* for two days. *Galleria* cadavers were then placed on White traps containing Ringer solution (Kaya and Stock, 1997) for a couple of weeks to collect emerging infective juveniles. The larvae solution collected is then washed with Ringer solution, stored at 15 °C and used for the experiments within 2 weeks.

Susceptibility of *Liriomyza huidobrensis* to entomopathogenic nematodes *in vitro*

The aim of this experiment was to determine if the *Liriomyza huidobrensis* pupae are susceptible *in vitro* to the EPNs larvae. For this purpose ten Petri dishes were filled with sterilized soil containing infestive juveniles-IJs of EPNs solution concentrated at 1000IJs/mL. Five pupae were added per Petri dish and placed in obscurity at 22±2°C. In total 50 pupae were used for each test. The experiment was repeated three times. In control Petri dish, *L. huidobrensis* pupae are added to autoclaved soil humidified with Ringer solution only. Due to the small size of *L. huidobrensis* pupae, they were considered as dead if no emergence of adult flies were observed. Pupae mortality was verified every 48hrs during 15 days which represented the duration time for adult flies emergence. Dead pupae were placed on individual white trap (Bedding and Akhurst, 1975) to harvest the emerging of IJs juveniles. Assessment of IJs emergence was done for each pupae cadaver during one month. IJs emergence were considered as an indicator for the success of the EPNs life cycle inside *L. huidobrensis* pupae.

### Results and discussion

Results of this study showed that following the EPNs application and out of the 150 pupae used in the three tests, of 16 ±1.5 % of the pupae emerged into adults of *L. huidobrensis*, 21±2.5% of the pupae were parasited by another natural pathogene for *Liriomyza*, (*Diglyphus isaea*) and 53±1.5% of the pupae were found dead, potentially infested by EPNs. The remaining pupae (10±1%) were not found in the Petri dish due to their small size. The control tests showed 79±2% of adult flies eclosion. Out of the 53±1.5% *L. huidobrensis* pupae cadavers potentially infested by EPNs, none of the cadaver showed any emergence of EPNs after one month eventhough the mummification and red color aspects of the cadavers (main signs indicating that an insect is parasited by EPNs).

Previous research on EPNs from the genus of *Steinernema* (*Steinernema carpocapsae* and *Steinernema feltiae*) demonstrated their potential for control of the agromyzid leafminers: *Liriomyza trifolii* Burgess (Hara et al., 1993; LeBeck et al., 1993; Sher et al., 2000; Tomalak et al., 2005) and *Liriomyza huidobrensis* (Williams and Walters, 2000). Results of this experiments showed leafminers larval mortality ranged from 48 to 98%.

Biological control using entomopathogenic nematodes offers an alternative approach for management of *L. huidobrensis*, because of its ability to rapidly develop resistance to chemical insecticides (Mason et al., 1987; Parella et al., 1989). Additionally, chemical insecticides are hazardous to the environment and non-target organisms. Entomopathogenic nematodes, in particular *Steinernema carpocapsae*, *Steinernema feltiae* and *Heterorhabditis bacteriophora* were already tested against different leafminers in the genus *Liriomyza* by several researchers (Harris et al., 1990; Olthof and Broadbent, 1990; 1992); *Liriomyza trifolii* Burgess larval mortality after application of *S. carpocapsae*, were 64% in laboratory (Harris et al., 1990) and 53 to 83% in greenhouse trials (Olthof and Broadbent, 1992), while with *H. bacteriophora* in laboratory trials, mortality were ranged from 76 to 90% (Olthof and Broadbent, 1990). Harris et al. (1990) showed significant reductions in leaf damage after *S. carpocapsae* treatment which encouraged further studies on the application of entomopathogenic nematodes against *Liriomyza*. In addition only few EPNs species were tested against *Liriomyza* and Bedding et al. (1982) showed the importance of selecting the appropriate species and strain of nematodes for each pest species. Accordingly we conducted laboratory experiment using an indigenous species of nematode, not tested previously against *L. huidobrensis* pupae. Our results showed an important mortality rate of *Liriomyza huidobrensis* pupae in laboratory conditions with no validation of EPNs reproductive cycle success inside the pupae (no EPNs infective juveniles emerged from pupae cadavers).

## Conclusion

The main challenge of using entomopathogenic nematodes in biological control programs are adaptation to local conditions such as temperature, predators, soil pH, that might help introducing EPNs in the soil (Klein, 1990). Using indigenous entomopathogenic nematodes may provide more suitable EPNs isolates for biological control uses because of the adaptation to local climate and population regulators of the insect pest (Bedding, 1990). In our study, *H. indica* indigenous strain is isolated along the coastal area of Lebanon, sharing the same environmental conditions as *L. huidobrensis*. Our results shown that this strain could cause a mortality of 53% of *L. huidobrensis* pupae, even without being able to achieve whole reproductive cycle inside of leafminer pupae. Accordingly, *H. indica* can be considered as potential agent in biological control regarding its capability to cause pupae mortality *in vitro* and being isolated in favorable environmental conditions to the presence of its host, *L. huidobrensis* pupae which could prevent field trial failures in further studies. Susceptibility of *L. huidobrensis* pupae against entomopathogenic nematodes under controlled conditions, as reported here, is the first step towards the development of an integrated pest management program. More studies are needed to validate the capacity of EPNs to penetrate *Liriomyza huidobrensis* pupae and to test *H. indica* against larval stages of *L. huidobrensis*. Our results could also have an ecological important component; being unable to reproduce completely inside *L. huidobrensis* pupae, the EPNs will be unable to persist in the environment and therefore the balance of the ecosystem will not be disrupted (Schroeder et al., 1994) and there will be no ecological risk on non target species.

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### **3. ORGANIC AGRICULTURE**

## GRAIN QUALITY IN ORGANIC AND ECOLOGICAL CROPPING SYSTEMS

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### Abstract

Ecological cropping includes combination of different crops at the same field and application of organic and mineral fertilizers, according to plant requirements. Organic cropping includes application of allowed organic fertilizers.

Trial was conducted during 2012. In ecological production maize and soybean were grown as: single crops (SC), in alternating rows (AR) and alternating strips (3 rows of each crop - AS). Fertilization regimes included: urea, Ofert (organic fertilizer), Uniker (microbiological fertilizer) and control. Organic production in trial included spelt, soybean and maize. Fertilization regimes were: DCM EKO-MIX 1 (F1), DIX 10 N (F2) and control. After harvest, grain yield, mass of 1000 grains, and content of phenolics, glutathione, phytate and  $\beta$ -carotene were determined in grain.

In ecological production the highest yields and 1000 grain weight were obtained in Uniker treatment, as well as with AR cropping in both crops, with two times higher values in soybean, in relation to control. Generally, soybean grain had higher levels of phytate, phenolics and  $\beta$ -carotene, compared to maize. In organic production differences in yield parameters were insignificant. Uniker show the highest impact on phytate and  $\beta$ -carotene accumulation in grain of both crops in ecological production, and the same trend was noticed in F1 treatment in organic production. The content of phenolics and glutathione varied among fertilization treatments, but the highest values were obtained in AR cropping. Lower level of phytate and higher level of  $\beta$ -carotene achieved in crops from organic production indicated higher nutritional quality of crops produced in this system.

**Key words:** antioxidants, cropping systems, fertilization, grain, composition

### Introduction

Ecological agriculture applies the strengths of natural ecosystems in agro-ecosystems, to produce food and fiber. The overall strategies include growing of healthy plants with good defense capabilities, stressing pests and enhancing populations of beneficial organisms (Magdoff, 2007). From that point, Ecological agriculture includes combination of different crops at the same field and application of organic fertilizers, enabling better utilization of space and nutrients, with minimal inputs. On the other hand, organic agriculture means production of high quality food or raw material with maintaining the soil quality. It is based on controlled input of allowed agrochemicals that are mainly natural products, like plant extracts, fertilizers originating from decomposed organic products or manure from organic cattle production.

Cropping systems aimed for high quality food production include all available measures that increase nutrients favourable for human health and growing of improved genotypes. For example, red maize provides 20% more protein than white or yellow maize. It is rich in anthocyanins and flavonoids, which are antioxidants (Žilić et al 2011a). Black soybean also contains two times more phenolics than yellow grain genotypes (Žilić et al., 2011b). Ruibal-Mendieta et al. (2005) emphasized higher nutritional value of spelt (*Triticum aestivum ssp. spelta*) than common wheat, with high content of mineral nutrients and low content of gluten and phytic acid. It is also rich in phenolics (Gawlik-Dziki et al., 2012).

One of the most important components for phosphorus storage is phytate (Schlemmer et al., 2009). Phytate is antinutritive, due to its indigestibility for monogastric organisms, but at the same time, has positive role as an antioxidant and anticarcinogenic agent. Malenić et al. (2007) underlined phenolics as bearers of antioxidative activity in soybean seeds, since the seeds with low phenolic's content have poor antioxidative activity, too. One of the most important antioxidative and nutritive factor is glutathione, which participate in signalling and stress prevention in plants (Foyer and Noctor, 2005). Grodstein et al. (2007) emphasized antioxidative function of  $\beta$ -carotene.

The aim of experiment was to compare ecological and organic production from the point of potential to improve grain quality of maize, soybean and spelt, based on content of antioxidants.

### Material and methods

Experiment with intercropping in ecological production was conducted during 2012, with varieties of red grain maize (variety Rumenka) and black grain soybean (variety Dukat). Crops were grown as: single crop (SC), alternating rows of both crops (AR) and alternating strips (3 rows of each species - AS). Fertilization regimes included incorporation of: Uniker (microbiological fertilizer applied 11 l/ha), Ofert (organic fertilizer applied 3 t/ha), urea (applied 163 kg/ha) and control (without fertilization).

Experiment with organic production included three fields. In the first field spelt wheat (variety Nirvana) was seeded in October of 2011. Second field included maize variety Rumenka with application of 2 organic fertilizers: F1 (DCM EKO-MIX 1 (N:P:K=9:3:3, 65% organic matter) and F2 (DIX 10 N (N:P:K=10:3:3, 72.5% organic matter) in amount of 500 kg ha<sup>-1</sup>, as well as control (without fertilization). Third field was seeded with soybean (variety Lidija). Spelt and soybean fields didn't include any treatment.

Table 1. Amount of nutrients, incorporated with different fertilization regimes

Fertilizer treatment	Fertilizer amount	Nutrient amount (kg/ha)		
		N	P	K
Uniker	11 l/ha		Microbiological fertilizer	
Ofert	3 t/ha	66	144	84
Urea	163 kg/ha	75	-	-
DCM EKO-MIX 1	500 kg/ha	45	15	15
DIX 10 N	500 kg/ha	50	15	15

Both experiments were set up in rain-fed conditions on chernozem soil type. After harvest, grain yield, mass of 1000 grains, and content of antioxidants (phytic acid (PA),  $\beta$ -carotene, phenolics and total glutathione (GSH)) were determined in grains. PA was determined by the method of Dragičević et al. (2011);  $\beta$ -carotene was determined according to AACC (1995) procedure; phenolics were determined by the method of Simić et al. (2004) and GSH by the method of Sari-Gorla et al. (1993). The obtained results were presented with standard deviation (SD).

Meteorological conditions during vegetative period of 2012 (Table 1) indicated unequal distribution of precipitation, with the lowest value in August accompanied with high average temperatures in July and August.

Table 1. Meteorological conditions during vegetative period of 2012

Month	IV	V	VI	VII	VIII	IX	Aver./
T average (°C)	14.45	17.90	24.56	27.08	26.21	22.14	22.05
precipitation (mm)	66.7	127.5	13.9	39.4	4	31.4	47.15

## Results and discussion

Our results in ecological production experiment underlined Uniker and AR as treatments with the highest achieved grain yield and 1000 grain weight of both crops, maize and soybean (Figure 1). Unfavourable meteorological conditions were particularly reflected on low soybean yield, irrespective to production type, where Uniker and urea in ecological, as well as in organic production induced negligible higher yields. On the other hand, ecological production brings upon increase in maize yield in Ofert treatment in AR (about 33% higher in regard to the same treatment in SC), while differences between treatments in organic production were minor. Undie et al. (2012) and Verdelli et al. (2012) also underlined that maize and soybean intercrops were more productive than sole crops. The differences between ecological and organic production in maize yield were about 2%. This corresponds with results of Messmer et al. (2009) who also reported small differences between grain yields in organic and conventional farming. The highest grain yield was attained with spelt, which avoided unfavourable conditions during grain filling period of maize and soybean (July, August).

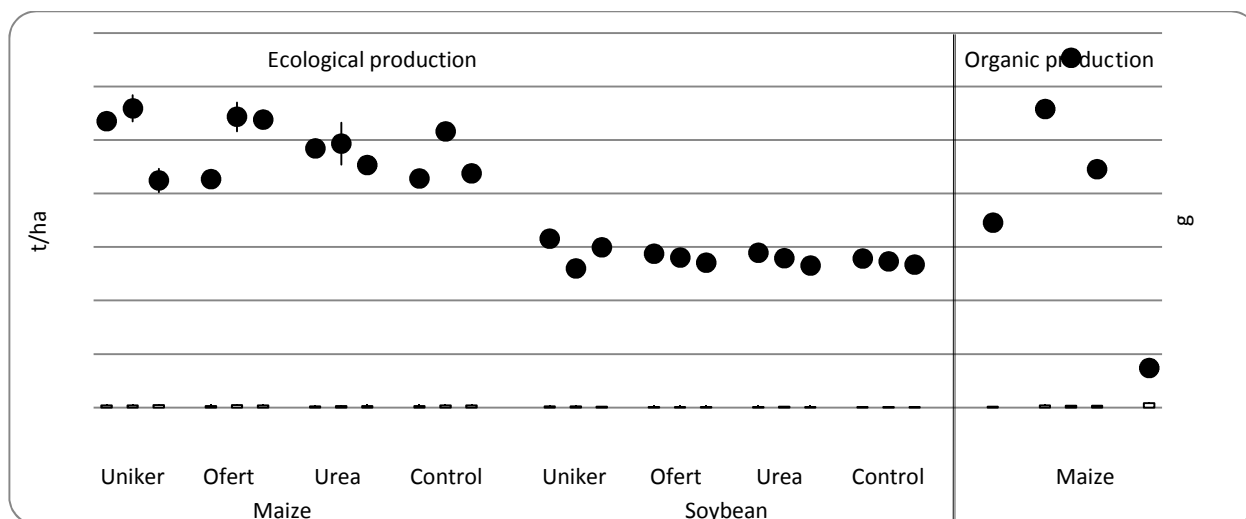


Figure 1. The influence of applied cropping systems (ecological and organic) and treatments on grain yield (□) and 1000 grains weight (●); SC – single crop; AR – alternating rows; AS – alternating strips; Cont. – control; F1 - DCM EKO-MIX 1; F2 - DIX 10 N; Mean value  $\pm$  SD.

Although phytic acid is antioxidant its low content in grain is desirable, since it obstructs absorption of mineral nutrients (Schlemmer et al., 2009). It is obvious that soybean has several times higher PA content in grain, compared to maize and particularly to spelt (Figure 2). In present conditions of ecological production, the lowest PA content was mainly noticed in maize and soybean grain at AR treatment, while the Uniker showed tendency to increase PA in grains, probably due to phosphorus nutrition enabled by different circumstances in rhizosphere (Zhang and Li, 2003). It was important to underline that average PA content was about 41% lower in maize grain from organic, compared to ecological production. The lowest PA content was observed in spelt grain, what could be its advantage as basis in foods with improved mineral availability (Ruibal-Mendieta et al., 2005, Queiroz et al., 2011).

Similar with PA, the highest  $\beta$ -carotene content was observed in maize and soybean grain from Uniker treatment, grown as single crop (Figure 2). It is also obvious that soybean grain has about 4 times higher  $\beta$ -carotene content than maize in ecological, while in organic production this difference was lower. F1 treatment increased PA and  $\beta$ -carotene contents in maize in organic production. Menkir and Mazya-Dixon (2004) noticed low variations in  $\beta$ -carotene content in grain

under the influence of environment, while the genotype was main source of variation. The lowest  $\beta$ -carotene content was in spelt grain.

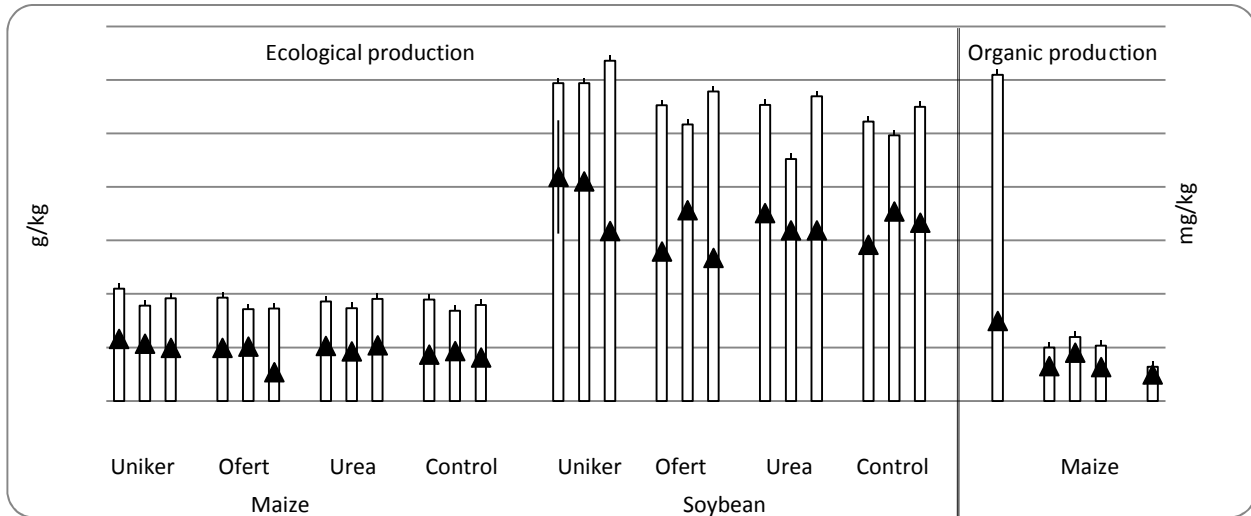


Figure 2. The influence of applied cropping systems (ecological and organic) and treatments on contents of phytic acid (□) and  $\beta$ -carotene (▲); SC – single crop; AR – alternating rows; AS – alternating strips; Cont. – control; F1 - DCM EKO-MIX 1; F2 - DIX 10 N; Mean value  $\pm$  SD.

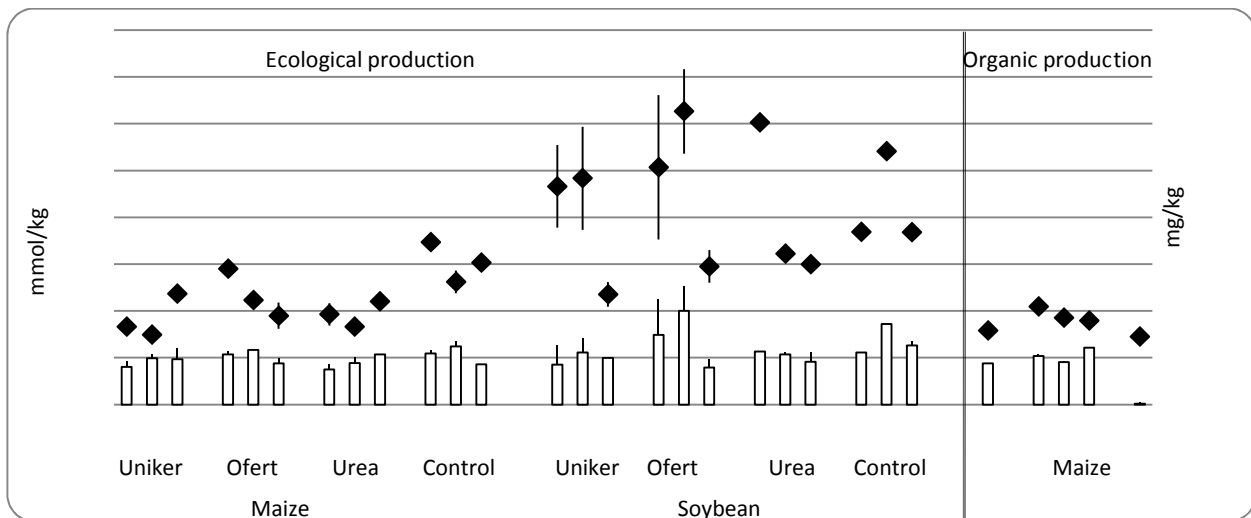


Figure 3. The influence of applied cropping systems (ecological and organic) and treatments on contents of total glutathione (□) and phenolics (◆); SC – single crop; AR – alternating rows; AS – alternating strips; Cont. – control; F1 - DCM EKO-MIX 1; F2 - DIX 10 N; Mean value  $\pm$  SD.

GSH is protein with important role in cell signalling and protection against oxidative attack (Foyer and Noctor, 2005). Ofert and AR were the treatments in ecological production that increased its content in maize and soybean grain (Figure 3), irrespective to its higher variations present in soybean. In organic production, GSH content was increased in F2 treatment. It is interesting that the lowest GSH content was obtained in spelt grain.

Phenolics are secondary metabolites, involved in protection from free radicals and pests (Santiago and Malvar, 2010). Their content varied the most (57-62%) among examined antioxidants (PA 19-29%;  $\beta$ -carotene 36-54%; GSH 39-57%) in grains from ecological production. The highest content of phenolics was observed in maize grain in control in CS treatment and in soybean grain in Ofert in AR treatment. Several times higher phenolic content was found in soybean from ecological

production, what could be attributed to its black grain. That could be important, since Malen ic et al. (2007) underlined that phenolics are bearers of antioxidative activity in soybean seeds. Relatively balanced phenolic content was in all three crops in organic production, with the highest level noticed in maize in control, while the lowest value was found in spelt. This value, obtained in spelt grain is in accordance with results gained by Gawlik-Dziki and Dziki (2012).

### Conclusion

Based on obtained results from preliminary research, it could be concluded that examined production models could increase nutritional value of maize and soybean grain. Alternating rows, as intercropping system of ecological production increased mainly grain yield and content of antioxidants (  $\beta$ -carotene, GSH and phenolics), as well as decreased PA level in maize and soybean grain. Both fertilizers, Uniker and Ofert improved yield and quality of produced grains, stressing Ofert as treatment which increased GSH and phenolics and reduced PA level. On the other hand, applied fertilizers in organic production didn't show expected results in maize yield and grain quality, while spelt was emphasized as low PA and moderate in phenolics grain.

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## ALBANIAN CONSUMER ATTITUDE AND BEHAVIOUR TOWARD ETHICAL VALUES OF AGRO-FOOD PRODUCTS

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### Abstract

It is of paramount importance to know consumers' behavior and attitude toward specific values linked to agro-food products. In fact, there is a constant increase of consumer concern on and interest in products bearing ethical values. The study aims at exploring Albanian consumer knowledge of and attitude toward agro-food products with ethical values such as organic, fair-trade and typical/traditional ones. The work is based on primary and secondary data. Primary information was collected by face-to-face interviews with 311 adult Albanian consumers concentrated mainly in the central, south-eastern, south-western and northern part of Albania. Respondents were contacted randomly mainly in markets and other shopping areas. Questions dealt mainly with sources of information, knowledge and attitude toward agro-food products with ethical values, including reasons and motivations for buying them, purchasing channels, as well as opinion about prices and willingness to pay. Results obtained show that Albanian consumers have positive attitude toward organic products. About 82% of the interviewees buy organic products because they link the term "organic" with products acquisition directly from farms or in farmers' market. Most of interviewed Albanian consumers (68%) are aware that their consuming behavior generates environmental, economic and social impacts. In the meantime, the main motivation for buying organic and typical products is that they are considered safer, healthier and tastier. Higher price and low market availability are the main obstacles that should be overcome in order to increase sales of agro-food products with ethical values, which will bring sustainable benefits to Albanian rural areas and consumers.

**Keywords:** consumer, agro-food, ethical values, Albania

### Introduction

The term ethical consumption - used for the first time in 1989 by the magazine for citizen activists in Manchester, "Ethical Consumer" (Elkington and Hailes, 1989) - now is becoming one of consumer's behavior tendencies. Ethical products and services are experiencing growing market shares and this phenomenon is not restricted only to Europe (Freestone and McGoldrink, 2008).

The main factors that helped developing this market are economical shift of post-industrial area (consumerism) (Martinengo, 2012), government policies and consumer information (Freestone and McGoldrink, 2008). Governmental policies in the past have contributed to ethical production and consumption through pollution control. These policies in Europe now have shifted from industrial pollution control towards bringing about more sustainable consumption patterns (Micheletti *et al.*, 2006). From the other side, consumers are becoming more aware of ethical consumption through market and information campaigns (Harrison *et al.*, 2005). Their behavior influences directly the type of products/services and way of production (Martinengo, 2012) because ethical trade and production have been both consumer and trade driven (Brown *et al.*, 2000).

Ethical consumption in the present paper refers to the consumer behavior of purchasing products and services produced in a way that minimizes social, animal welfare and/or environmental impacts, while avoiding products and services considered as having a negative impact on society, animal welfare and environment. Ethical consumer as a term represents a further development of the term “green consumer” defined by Elkington and Hailes (1989). Ethical consumption compared to “green consumerism” encompasses a broader range of ethical issues including matters of conscience such as animal welfare and fair trade, labor standards, self-interested health concerns (Cowe and Williams, 2000), their concern for deep-seated problems such as those of people of the third world (Shaw and Clarke, 1999), whether a corporation promotes people of minorities, worry about product transportation distances, etc. (Harrison *et al.*, 2005).

The shift of today consumers toward values of social responsibility can be explained by Maslow’s hierarchy of needs, and corresponds to self-actualization and self-fulfillment (Simons *et al.* 1987). The last shift of consumer’s behavior needs to be further explored by research.

The objective of this research is to explore Albanian consumer’s perceptions, knowledge, beliefs, attitudes and behavior toward ethical products such as fair trade, organic and traditional or local ones.

There is very few literature and research on marketing and Albanian consumer’s behavior toward agri-food products. To the best knowledge of authors of the present paper, studies dealing with Albanian consumer’s behavior refer to individual products such as, wine (Civici *et al.*, 2004), lamb meat (Imami *et al.*, 2011), olive oil (Chan-Halbrendt *et al.*, 2010), table olives (Zhllima *et al.*, 2012), apple fruits (Skreli and Imami, 2012) and milk (Kapaj *et al.*, 2013). They take into consideration the different attributes of products in general and no one of them has studied consumer behavior, knowledge, perceptions and attitude toward ethical products.

### **Materials and methods**

The present study was based on secondary and primary data: secondary data consist on literature review while primary data on face-to-face questionnaires and interviews with key persons of the sector in Albania. Questionnaires were exercised to 311 Albanian adults in Albanian urban areas. They were filled out by consumers in four main areas of Albania with the scope of including different economic and social distinguished characteristics: central Albania (Tirana, Kruja and Durrës), northern part (Tropoja and Kukës), south-western part (Berat) and south-eastern part (Gramsh).

The questionnaires had three main sections: a). demographical and economical data; b). consumer’s behavior in the market; and c). knowledge, beliefs, motivations and attitudes toward ethical products. The first section (a) was related to demographic and economic information like: age, gender, level of education, employment, marital status, and size of family, children and elder presence, family monthly income. The second one (b) dealt with questions such as: what does ethical product mean; what type of product they buy; on what basis, how often, and where they buy; whether they read and what they search in the label; willingness to pay; types of products they would prefer; to what extent and frequencies would they buy, etc. The last section (c.) asked respondents about: what is the contribution of organic, fair trade and local/typical products to ethical issues such as the environment protection (decreasing food miles and emissions, biodiversity and ecosystem conservation, better natural resource use, etc.), consumer’s impact on animal health and welfare, social and civic impacts (food quality and safety, safe and equitable workplace, gender equity, transparent and trustworthy food systems, civic responsibility and care, human rights), economic impacts (fair and equitable financial returns for local farmers/producers, availability of ethical products and affordability of consumers), sources of information for ethical products, knowledge about their certification and control systems, their willingness to be informed further and which type of channels they would prefer, reasons for buying or not buying ethical products).

The respondents were chosen randomly from the end of 2011 till December 2012. The sample belongs to different ages, genders, educations and origins, and family statuses and sizes. Respondents consisted of respectively 58% males and 42% females. Most of them were married (71%); 58.7% of interviewees had children; 91.9% had at least secondary school level and 44.1% graduated at university (5.1% had postgraduate studies). Like in Italian and Serbian studies carried out by Driouech *et al.* (2010, 2013), the range of age intended was from 18 to 50 and over: 65.5% of respondents belong to the age range between 31-50 and over, the age of those that deal more frequently with household shopping. Most respondents (66%) were active (53.5% were employees and 12.5% business-runners); 25.8% were students and housewives and only 13.4% were retired.

Tab. 1. Respondents profile characteristics (n=311)

Items		Percentage (%)	Items		Percentage (%)	
Gender	Male	58.0	Family size	Till 2 members	11.1	
	Female	42.0		3 members	11.1	
Age	18-24	19.0		4 members	32.5	
	25-30	15.0		5 members	24.4	
	31-40	18.0		6 members	13.0	
	41-50	21.0		More than 6 members	7.8	
	51 and over	27.0		Student	15.0	
Level of education	Primary school	8.50		Employment**	Employee	52.4
	Technical/professional school	37.8			Businessman	11.0
	Secondary school	39.0			Housewife	8.2
	College/University	10.0	Retired		12.2	
	Postgraduate (Master, PhD)	5.1	Unemployed		1.2	
	Other	0.00	Family income (ALL/Month)		Less than 20 thousand	7.2
Family status	Single	29.0		21-40 thousand	26.5	
	Married	12.3		41-60 thousand	33.7	
	Married with children	58.7		61-100 thousand	22.3	
Household composition**	Children	77.7		More than 100 thousand	10.2	
	Elders	47.0	*: . Percentage greater than 100, due to multiple choice			
	People with health problems	6.0	**: ALL: Albanian Lek, 1 €=140 ALL.			
	None of above	12.0				

Data collected through questionnaires were analyzed with descriptive statistics using Microsoft Excel program.

The present study was based on following hypothesizes: Albanian consumers have already basic information on ethical products such as organic, fair-trade and typical/local ones; they are aware of and committed to benefits and positive impacts of ethical products for human health, environment and social justice and; there is more space and willingness to pay for ethical products in the Albanian market.

### Results and discussion

About 82% of the Albanian interviewees buy organic products and 50% of them buy them with weekly frequency (20% everyday and 30% 2-3 times a week). It was noticed that “organic” products, for Albanian respondents, mainly mean products acquired directly from farms or in farmers’ market. They do not link it with organic standard and certification. It was noticed that most of Albanian consumers associate between the terms “ethical”-“organic”-“natural” and “farmer’s” products. Ethical products that respondents buy more are “organic”: olive oil (50%), fruits (47%), cereals and pulses (40%), processed food (39%), vegetables (35%) and meat (31%). As previously mentioned, Albanian consumers associate the term “organic” with farm and farmer’s market. For

example, Albanian consumers prefer to buy olive oil directly from olive mills supplied with olives of small farmers (Albanian Association of Olive Oil, 2012). For 75% of Tirana consumers, the domestic originated olive oil has the most important attribute (Chan-Halbrendt *et al.*, 2010). Preference for products of domestic origin as a product attribute is found also for wine (Civici *et al.*, 2004), table olives (Zhllima *et al.*, 2012), apple (Skreli *et al.*, 2012), for milk as a second attribute after fat content (Kapaj *et al.*, 2013) and meat (86% of interviewees preferred meat from butcheries) (Imami *et al.*, 2011). Reason for buying domestic and local products can be explained by the impact of scandals and misinformation. Larger value chains are distancing consumers from production systems, increasing misinformation about the products safety and ethical issues (Corcoran *et al.*, 2002).

Regarding places where they buy ethical products, the most important indicated place was the supermarket, answer that brings contradiction with their belief that “organic” is “farm” product. This is supposed to happen for different reasons: consumers tend to buy the highest share of important commodities such as cereals, fruits, milk, oil etc. of local origin and mostly directly from farmers or their markets; they buy the same locally originated products in the supermarket, too, adding to them during shopping other commodities that are processed, packed or industrialized origin.

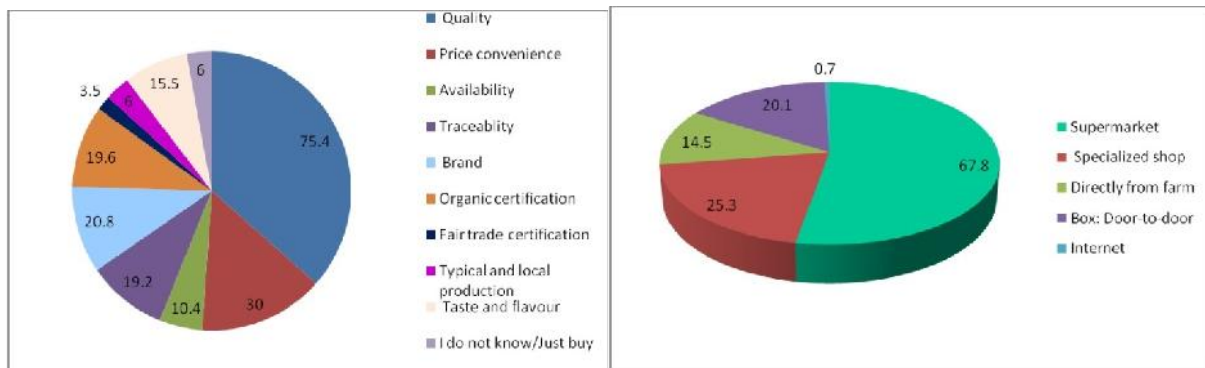


Fig.1. Places where consumers buy ethical products\* Fig. 2. Reasons for buying ethical products\*

\*: Percentage greater than 100, due to multiple choices

The most important attributes of products considered by Albanian consumers are: quality (75.4%) followed by price convenience (30%), brand (20.8%), organic certification (19.6%), traceability (19.2%) and taste and flavor (15.5%) (Fig. 2).

Together with the quality of the products, price is one of the most important attribute that influence the consumer's behavior in Albania as demonstrated by other studies for different products. According to those studies done recently, Albanian consumers together with the origin consider important the price of products (Chan-Halbrendt *et al.*, 2010; Imami *et al.*, 2011; Skreli *et al.*, 2012; Zhllima *et al.*, 2012; Kapaj *et al.*, 2013). Except olive oil where the higher price is indicator of quality inducing higher selling, the other products studied in Albania, are preferred less in case they are highly priced (Imami *et al.*, 2011; Skreli *et al.*, 2012; Zhllima *et al.*, 2012; Kapaj *et al.*, 2013). Behavior of Albanian consumers against olive oil price and its identification with quality can be explained with asymmetric or evasive information (Standalidou *et al.*, 2002; Cicia *et al.*, 2002). When respondents were asked particularly to compare price of ethical products with conventional ones about 52% of them think is medium and 45% think is high. This can explain why Albanian consumers give low share to attributes organic, fair-trade and typical certified compared to quality and price even think that ethical products have better taste. Regarding taste attribute, about 83% of respondents have the belief that ethical products (particularly organic and local products) have a better taste; about 15% of respondents think that ethical products have the same taste as conventional products and only about 2% think that the taste of ethical products is not as good as

conventional ones. When asked for reasons of not buying ethical products, respondents gave two main reasons: the price (56% of respondents) and the availability (about 38%). However, most of the respondents are ready to pay a premium price for ethical (=organic) products: about 91% of them could pay more for ethical products; 25% of them up to 10% higher price; 32% of respondents 11-30% more, 19% could pay 60% more and 14% of respondents could pay 31-60% more.

For the question regarding labels, about 93% of respondents answered that they read label before buying; about 86% of them read expiry date (linked to food safety), about 30% the origin of the product, about 25% ingredients, about 20% nutritional ingredients and only 15% quality certifications.

As for the meaning of the term “ethical” product, about 44% of them answered “agro-food produced organically”, about 22% agro-food products produced locally, 20% production that respects the environment, about 19% agro-foods produced by farms involved in ethical activities, 19% any agro-food production. Only 13% of respondents answered that ethical products are those that respect producers and workers.

Albanian consumers (68%) are aware that their consuming behavior generates environmental, economic and social impacts. About 76% of respondents answered positively the question whether they have knowledge on ethical products, 16% of them responded maybe and only 7% of them responded no.

These answers are in contradiction with answers for another question on how they can generate impacts with their consuming behavior. About 100 respondents (or 30%) skipped the question; the rest mostly answered that the consumer behavior influences less or enough environmental emissions, ecosystems and biodiversity, animal welfare and so on. In the meantime, the main motivation for buying organic and typical products is that they are considered safer, healthier and tastier.

The main source of information regarding ethical products has been electronic mass media including internet, TV, radio (about 58%), lessons in school and training sessions (about 41%), friends (about 24%), newspapers and magazines (about 17%) and lastly stores and shops (16%).

Respondents were asked whether they were interested to know more and be informed about ethical products. Almost all of interviewees responded that they were interested (about 96%).

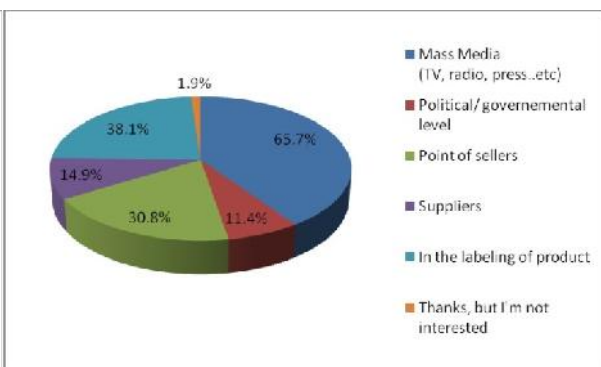
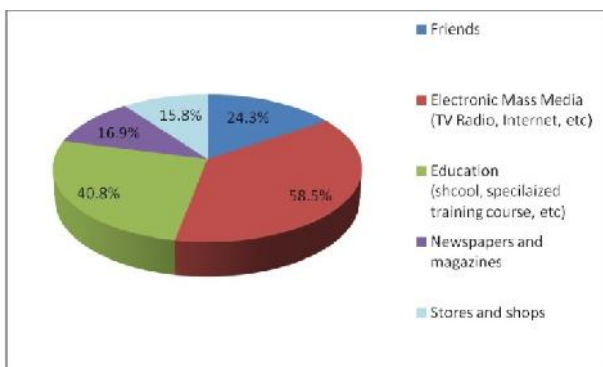


Fig. 3. Sources of information for ethical products\*  
\*: . Percentage greater than 100, due to multiple choices

Fig. 4. Recommended information channels\*

The view about possible channels to communicate with consumers about ethical products is as following: mass media (about 65.7%), labels (38%), selling points (about 31%), suppliers (about 15%) and government (11%) (Fig. 4).

### Conclusions

The main conclusions drawn at the end of the present research are:

- Most of Albanian consumers have positive attitude regarding ethical products because 81% of them think that are healthy; they think ethical products mostly are produced taking care for the environment and have not much motives related to social impacts;
- For Albanian consumers, ethical product, mostly is organic and local specially from farm and respective zone; they think that they buy ethical (=organic) products every day or week either in farmers' market or door-to-door products;
- The main motive for buying ethical (organic) products is because they are healthier and safer; they buy in the market mostly based on quality and price and the main reasons for which Albanian consumers do not buy ethical products are their higher prices and their unavailability especially in supermarkets; however, about 91% of them are ready to pay a premium price for organic products;
- Even most of Albanian consumers (76%) declare that they have knowledge about ethical products, they want to know more about ethical products (96%) and prefer: mass media, selling points and suppliers, and governmental initiatives.

Overall, higher price, low market availability and education are the main obstacles that should be overcome in order to increase sales of agro-food products with ethical values. The latest could bring sustainable benefits to Albanian rural areas and consumers. Public policies can have an important role especially supporting education and ethical food production and consumption initiatives. Further studies should be carried out including application of a logit model to link better demographical and economical characteristics of Albanian consumers to attitudes and behavior.

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## EFFECT OF FOLIAR NUTRITION ON MORPHOLOGICAL CHARACTERISTICS AND SOYBEAN YIELD IN ORGANIC CROPPING SYSTEM

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### Abstract

Investigation was conducted with the aim of soybean grain productivity determining in the organic cropping systems on parcel of the Institute of Field and Vegetable Crops, Backi Petrovac (N45°20', E19°40'89msl). The study object was the Galina variety, 0 maturity group. The effect of foliar nutrition on morphological characteristics and soybean yield was studied. The experiment included two variants: variant without fertilizing (control) and fertilized variant (Slavol was used in fertilized variant).

The average soybean yield in the organic cropping system was 4,622 kg/ha. 1,000 grain weight averaged 173 g. The yield was higher by 258 kg/ha or 5.74% in the fertilized variant and 1,000 grain weight was higher by 13.2 g or 7.9% compared with the control.

The plants height in the treated variant was significantly higher compared with the control ( $p < 0.5$ ).

The plants had on an average 81.94 cm stem height and average plant weight was 16.00 g.

Foliar nutrition showed to be a feasible method of soybean productivity improving in organic cropping system.

**Key words:** Glycine max, organic cropping, morphological characteristics, yield, foliar nutrition

### Introduction

In the early 1900s soybean was a little – known plant that was grown in only a handful of countries, but by the end of the 20th century it had developed into a leading field crop on the global scale. Nowadays, soybean is grown in most countries to a greater or lesser extent, but in recent years about 90% of the world's production have been concentrated in only several countries. Contribution of Europe to world soybean production ranged from 1 to 2%. Soybean (*Glycine max* (L.) Merr.) is an erect annual plant with a hairy stem reaching 30 cm to 130 cm in height depending on environmental factors. Pod contains one to five seeds what is affected by the environmental factors. The soybean plant has many varied usages and importance is multifaceted. The most important components of soybean seed, proteins (about 40%) and oil (about 20%) are the main reasons for soybean cultivation. High soybean yields require the harmony of all production factors. In practical terms, that means the right choices in plot selecting and the most suitable crop rotation, then timely applied tillage measures, fertilizer, necessary weed control, and finally, promptly and efficiently crop harvesting (Miladinovic et al., 2011).

Soy is suitable for growing in organic production. Organic farming put emphasis on the control, quality and safety of the produce, along with the application of ecological principles. Control over the production of organic soybeans is required so customers could obtain high quality produce. High yields and grain quality (Popović et al., 2012) prove that soybeans can be successfully grown in organic production if proper zoning and proper production technology are applied. Organic farming maintains and improves soil fertility in the long run. Soil fertility is maintained by a

production system - crop rotation harmonized with proper soil cultivation, fertilization based on soil fertility level (organic and other permitted fertilizers), and other cultural practices (Bavec, Bavec, 2006).

Organic products are safe because they are subjects of regular production process control, certified production is transparent and traceable, and documents and the logos are guarantee that food is organically produced (Lazic, Lazic, 2008). By organic production methods applying, the level of agricultural soil fertility and resources are protected, preserved and increased. This approach requires continuous planning and multidisciplinary. There are on an average about 30% more butterflies, soil microorganisms, earthworms, insects and various small mammals (Bengtsson et al., 2005) on farms engaged in organic production compared with conventional one.

The aim of this study was to examine the affect of foliar nutrition on soybean productivity by following the organic growing system.

### Material and method

The experiment was carried out on the experimental field of Institute of Field and Vegetable Crops in Ba ki Petrovac, in 2012. The soil type in the experimental plot was chernozem. Soil on the experimental parcel was of weak alkaline reaction (pH in KCl = 7.48), with lot of humus, 2.42%, medium provided with nitrogen, 0.184%, highly provided with available phosphorus (33.7 mg/100 g soil) and well provided with potassium (20.5 mg/100 g of soil). The experiment was done with an NS soybean cultivar, maturity group 0, by usage of organic farming methods, and it was established according to the split plot method with four replications. The experimental unit size was 10 m<sup>2</sup>, with the row spacing of 50 cm. The preceding crop was wheat. Primary tillage (deep plowing) was completed on November the 12<sup>ve</sup>, 2011. Manure was dismantled in the amount of 15 t / ha, on November the 11<sup>th</sup>, 2011. Seedbed preparation was done on March the 24<sup>th</sup>, 2012 and April the 30<sup>th</sup>, 2012. The soybean cultivar Galina was grown in the stand of 500,000 plants/ha. Soybean sowing was performed on May the 5<sup>th</sup>, 2012. Seed inoculation with NS Nitragin, a microbiological preparation, was done immediately before sowing. The crop was irrigated 2 times with 35 liters of water per m<sup>2</sup>, in July and August.

The experiment included two variants:

- 1 Control variant, without foliar nutrition
- 2 Nutrition variant, with foliar nutrition (only Slavol)

The Slavol, is organic microbiological fertilizers, product Agrounik d.o.o., Serbia, – a foliar nutrition was applied 2 times in the concentration of 60 ml / 10 l of water at the reproductive stage (R1 and R2) season.

Harvest was carried out by hand, in technological maturity. After harvest, samples were measured for moisture content. The yield was calculated per unit area, on 13% moisture basis. Following parameters were measured on each parcel: the height of 10 plants, plant weight, 1,000 grain weight and first pod height.

The analysis of the experimental data was performed by descriptive and analytical statistics using the statistical package STATISTICA 10 for Windows. The analysis of variance was done with one variability factor (nutrition). Significance of results was estimated by the LSD test for significance levels of 0.5% and 0.1%.

**Meteorological conditions:** Data for the analysis of the weather conditions were used from the meteorological station of Ba ki Petrovac, Serbia. During the 2012 growing season, the mean monthly temperature was 20.68°C; 2.80°C higher than the long-term average for Ba ki Petrovac. The rainfall was 221.5 mm, 200 mm less than the long-term average (Graph. 1).

The main limiting factor in the 2012<sup>th</sup> was the lack of water in the soil in most of the growing season and high temperatures, especially in June, July and August (23.4 °C, 25.2 °C and 24.5 °C), Fig. 1.

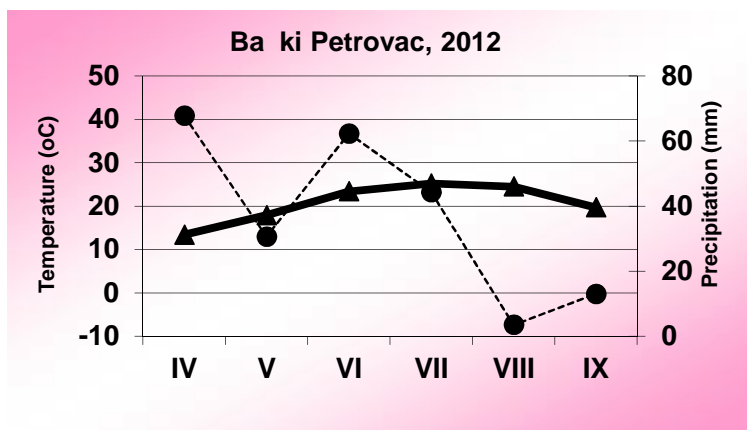


Fig. 1. Average air temperature and precipitation, Ba ki Petrovac, 2012

On the studied location, the amount and distribution of rainfall during growing season exert the decisive influence on soybean yield (Popovic et al, 2012, 2012a, 2013).

### Results and discussion

**Grain yield and 1000-grain mass of NS soybean.** The average yield of soybean had an average of 4,622 kg/ha and had high stability,  $C_v = 3.94\%$ . The average 1,000-grain mass of soybean had an average of 173.73 g and had high stability,  $C_v = 5.37\%$ , Table 1. The results showed that the foliar nutrition variant had a higher grain yield than the control (4751 kg/ha and 4,493 kg/ha, respectively), however, the difference was not statistically significant ( $p > 0.5$ ). The average yields in the foliar nutrition variant were higher by 258 kg/ha or 5.74 % compared with the control and 1,000-grain mass by 13.2 g or 7.9 % (Table 1, 2). Foliar nutrition proved to be a feasible method for increasing the grain yield and 1,000-grain mass of soybean.

Table 1. Yield (kg/ha) and 1,000 grain mass (g) of NS soybean in organic cropping system

Parameter	Genotype	Control	Nutrition	Average	Rate of change	$C_v$
Yield	Galina	4,493.33	4,751.33	4,622.33	5.74	3.94
1,000-grain mass		167.13	180.33	173.73	7.89	5.37

Parameter		Yield	1,000-grain mass
LSD test	0.5	481	21.79
	0.1	799	36.14

Table 2. Descriptive Statistics for yield and 1000 grain mass of NS soybean

Descriptive Statistics							
Effect	Level of Factor	Valid No	Mean	Confidence -95,00 %	Confidence +95,00 %	Std. Dev.	Std. Error
<b>Yield</b>							
Total		6	4,622.33	4,373.67	4,870.99	236.95	96.73
Variant	Control	3	4,493.33	4,247.94	4,738.73	98.78	57.03
Variant	Nutrition	3	4,751.33	4,044.73	5,456.93	284.04	163.99
<b>1,000-grain mass</b>							
Total		6	173.73	161.94	185.53	11.24	4.58
Variant	Control	3	167.13	140.72	193.55	10.63	6.14
Variant	Nutrition	3	180.33	159.25	201.41	8.48	4.89

Our results were in agreement with those of Senevirante et al. (2000) who reported that seed inoculation and fertilizer application increased grain yield of soybean.

#### Soybean morphological characteristics.

The average plant height of soybean had an average of 81.49 cm and had huge stability,  $C_v = 3.01\%$ . The results showed that the foliar nutrition variant had a higher plant height than the control (83.69 cm and 80.20 cm, respectively); however, the difference was statistically significant ( $p < 0.05$ ), Table 3.

Table 3. Morphological characteristics of NS soybean in organic cropping system

Parameter	Genotype	Control	Nutrition	Average	Rate of change	Cv
Plant weight, g	Galina	15.29	16.72	16.00	9.35	6.31
Plant height, cm		80.20	83.69	81.94	4.35	3.01
First pod height, cm		17.88	15.33	16.60	-6.48	4.74

Parameter		Plant weight	Plant height	First pod height
LSD test	0.5	4.20	2.49	7.93
	0.1	6.90	4.14	13.16

The plant weight was 16 g. The plants had on an average first pod height of 16.00 cm and had stability, Table 3, 4. The results showed that the foliar nutrition variant had a higher plant weight than the control (16.72 g and 15.29 g, respectively), however, the difference was not statistically significant ( $p > 0.5$ ), Table 3, 4.

Table 4. Descriptive Statistics for morphological characteristics of NS soybean

Descriptive Statistics							
Effect	Level of Factor	Valid No	Mean	Confidence -95,00 %	Confidence +95,00 %	Std. Dev.	Std. Error
<b>Plant weight</b>							
Total		6	16.00	14.08	17.94	1.84	0.75
Variant	Control	3	15.29	13.04	17.54	0.91	0.52

Variant	Nutrition	3	16.72	10.59	22.84	2.46	1.42
<b>Plant height</b>							
Total		6	81.94	79.68	84.20	2.15	0.88
Variant	Control	3	80.20	78.08	82.32	0.85	0.49
Variant	Nutrition	3	83.69	80.46	86.92	1.30	0.75
<b>First pod height</b>							
Total		6	16.60	13.00	20.21	3.43	1.40
Variant	Control	3	17.88	12.06	23.70	2.34	1.35
Variant	Nutrition	3	15.33	4.46	26.19	4.37	2.53

The average plant weight in the foliar nutrition variant was higher by 1.43 g or 9.35 % compared with the control and plant height by 2.83 cm or 3.5 % (Table 3, 4).

The average first pod height in the foliar nutrition variant was lowest by 2.55 cm or 16.6 % compared with the control, (Table 3, 4).

Based on the investigated results, it was found that soybean produced in organic production system achieved good yields in the year bad for the production of seed crops.

Foliar nutrition proved to be a feasible method for increasing the grain yield and 1000-grain mass, plant weight and plant height of soybean, what is consistent with the results of other researchers (Popovic et al, 2011, 2012b, 2013). Popovic et al. (2011) reported obtaining significantly higher yields of soy grain as a result of foliar dressing with an increased nitrogen fertilizer rate.

Peele (1997) reported that the foliar uptake of macronutrients increased the yield of soybean grains by 30 to 400 kg/ha. In a study by Oko et al. (2003), the foliar application of urea in an early reproductive stage (R2-R3) increased the grain yields of soybean by 6-68%. At Randjelovic's et al. (2006), mineral nutrients had a positive effect on soybean yield levels. The differences in grain yield were statistically significant, with an average increase of 540 to 1,690 kg/ha (20-62.22%) compared with the treatment in which no mineral nutrients were added.

### Conclusion

The results obtained in the present study have led to the following conclusions:

The average soybean yield was 4,622 kg/ha and average 1,000-grain weight was 173 g. The results showed that the foliar nutrition variant had a higher grain yield, 1,000-grain mass, plant weight and plant height than the control. The yield in the treated variant was higher by 258 kg/ha or 5.74% and 1,000 grain weight was higher by 13.2 g or 7.9% than in the control.

The plant height in the treated variant was significantly higher than in the control ( $p < 0.5$ ).

Foliar nutrition showed to be a feasible method for soybean produced in organic farming system productivity improving.

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## **PARTICIPATORY PROCESSES OF AGROECOLOGICAL INNOVATION IN ORGANIC CEREAL BREEDING: A CASE STUDY FROM ITALY**

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### **Abstract**

The growing interest towards organic and low input agriculture in Europe has highlighted the lack of cereal breeds suitable for these farming systems and their markets. To overcome this problem, new approaches to cereal breeding have been proposed, known as Participatory Varietal Selection and Participatory Plant Breeding. Based on the adoption of cereal's landraces and old varieties, these methods involve farmers, researchers and food processors with a participatory method. In this article we analyse the reasons and the implications of this approach, interpreting it as a case of open innovation, allowing access to, absorption and exploitation of external knowledge, with liberation of expertise for other members of the cereal supply chain. Emphasis is given to the important social signalling value and the general implications of this practice: the democratisation of the food system. Another consequence of this approach is the establishment of new organizational structures of innovation processes in agriculture, which can also be applied to other breeding methods. The next step could be the inclusion of consumers into the cereal breeding practice, in order to include their preferences and customs right from the beginning of the process. The article is completed by a case study of an organic cereal farm in Italy, part of the European project SOLIBAM, which is aimed at developing new strategies for organic and low-input integrated breeding. The case study is analysed within the framework of AE and open innovation paradigms, in order to understand participatory breeding practices and the consequences of their diffusion.

**Keywords:** plant breeding, organic cereals, agroecology, participatory approach, open innovation.

### **Introduction**

Italy is an important producer of organic products and the fourth most important market in Europe (SINAB, 2012), but still the availability of organic seeds is not sufficient for many important crops and there are not enough varieties available; although organic agriculture (OA) is well established in most European countries, only little attention has been given to breeding programs specific for organic farming systems. The cropping conditions of OA require varieties especially selected, capable to compete with weeds, to resist to pest and diseases as well as to difficult climate conditions, and to develop an expanded root system. Moreover, varieties for OA should have a broad range of environmental adaptability, to cope with the large variability of environmental conditions (Wolfe M.S. et al., 2008); in fact modern plant breeds have been selected for a broad geographical adaptability, which is exactly the contrary of what peasants did for millennia (Ceccarelli, 2009). The limited availability of organic seeds and the lack of breeding programs specifically aimed at satisfying the needs of OA, has recently stimulated new projects to support public research in this sector. The European project SOLIBAM - acronym for "*Strategies for organic and Low Input Integrated Breeding and Management*" - aims at developing new approaches to plant breeding and to cropping systems, in order to increase quality, sustainability and reliability of organic and low input productions, both in Europe and in Sub-Saharan Africa. In Work Package 6 (*Participatory Plant Breeding (PPB) and Management*) specific strategies for participative research in plant breeding are developed. As suggested by Wolfe M.S. et al. (2008),

the need to select for particular adaptability in target environments can receive a positive contribution from decentralized breeding with farmer participation and the use of crops buffered by variety mixtures or populations. In all breeding programs we can find four common phases: 1) generating genetic variability through crosses, mutations or the introduction of exotic germplasm; 2) selecting the best genetic material out of all the genetic variability obtained; 3) evaluating and comparing the selected lines with the existing cultivars, in trials that can take place in experimental fields or on farm. While in conventional breeding all the decisions are taken by the team of scientists, in PPB programs the end-users are involved in the decision making process as soon as possible, generally from the second phase. PPB can be thus defined as a process with the involvement of several partners (farmers, traders, consumers, breeders, researchers) from the early stages of breeding programs, taking full advantage of the complementarity of skills and knowledge from each partner (Wolfe et al., 2008). The main features of PPB, compared to conventional breeding, are: a) experimental trials are carried out on farms and are managed by farmers; b) farmers participate equally with breeders to the process of selection; c) the process may be repeated in an independent way in a large number of countries and areas, with different methods depending on the crop and the country (Ceccarelli, 2009). PPB has been applied until now in marginal and disadvantaged environments of Developing Countries. OA in Europe often occurs in marginal environments (Bishaw & Turner, 2007), and the limits of conventional breeding have become increasingly evident; this led to a growing interest for PPB also in Europe, where a pilot project was started in 2001 by INRA in Montpellier, France (Desclaux, 2005). Moreover, due to recognition of the negative externalities of CA (both for the environment and human health), and of the new challenges for the future (food security issues, biodiversity protection, climate change, etc.), there has been also an increasing interest in new agricultural paradigms, like agroecology (AE). Olivier De Schutter, the UN Special Rapporteur for the rights on food, suggested AE as a successful approach to face the actual food, environmental and energetic crisis in agriculture (UN, 2010). AE has been initially defined as the application of ecological principles to agriculture (Altieri, 1983); lately it became an interdisciplinary field of studies, including several scientific disciplines, agronomic practices, social and political movements (Wezel et al., 2011). The participatory approaches to plant breeding, like PPB, are an example of successful agroecological practices.

### **Materials and methods**

The methodological framework used for this paper was qualitative research approach; data have been collected through literature review research, interviews (see table 1) and participation in the annual meeting of the Italian Rural Seeds Network (RSN) and the SOLIBAM project. The participant observation method was also adopted in 2011 and 2012 during the “PPB Week” at *Pratini* farm (Italy). Therefore it may reflect our bias and subjectivity (Kumar, 2011).

The choice of this case study is justified due to the fact that the farm is carrying out innovative strategies in organic cereal breeding, together with the first PPB trials in Italy. AE was chosen as a framework for our paper because it underlines the importance of the link between science and society, and of the contribution of professional, traditional and local knowledge by actors, which usually do not participate in decision making and innovation processes.



**Table 1**

<b>Actors interviewed</b>	<b>Role and activity</b>	<b>Form of the interview</b>
Rosario Floriddia	Farmer and owner of <i>Pratini</i> farm	semi-structured
Riccardo Bocci	Coordinator of RSN	structured
Riccardo Franciolini	Staff member of RSN	semi-structured
Claudio Pozzi	Collaborator of RSN	informal
Ambrogio Costanzo	PHD candidate with thesis about PPB	semi-structured
Stefano Benedettelli	Professor, Florence University	structured

### **Case study description**

The organic farm *Pratini* is situated in Tuscany region (Italy), and more precisely in Pisa province. Rosario Floriddia and his brother Giovanni own 300 Ha of hilly land with clay soils; major crops are cereals (wheat, barley, oat, and millet), legumes (chickpeas, lentils) and fodder (alfalfa, clover, faba bean *var. minor*). The farm turned to OA in 1987, mainly to face the *price squeeze*<sup>7</sup> by cutting off the external chemical inputs. In 2006, following the suggestion of the President of “Tuscan Coordination of Organic Producers” (CTPB), they started to cultivate older varieties of bread wheat, acknowledging that the modern ones were not suitable for OA cropping conditions. Due to the support of the Universities of Pisa and Florence, and to the good agronomical and economical results obtained, since 2009 *Pratini* farm grows only older wheat varieties and landraces, and started carrying out different breeding trials with participatory approaches within the SOLIBAM project.

**Participatory Varietal Selection (PVS).** PVS is a selection process in which farmers and other partners are involved in the last phase of the breeding program, to evaluate and select the best varieties previously obtained by researchers (Ceccarelli, 2012). In collaboration with the University of Florence, several trial plots of old bread wheat varieties were planted at *Pratini* farm, and a decentralized PVS process was applied to determine the best varieties suitable for artisanal bread production. A blend of these varieties is now used at the farm to produce bread, and it is re-sown year after year. All the old varieties are also cultivated separately in single plots (1,5 x 10 m), in order to provide the farm with its own seed bank. Other experimental plots were sown with populations of durum wheat, obtained by the University of Florence from crosses between modern varieties (Urria, Svevo, etc.) and the old Italian variety *Senatore Cappelli*; this variety is appropriate both for OA and pasta production, but at *Pratini* farm it didn't give good agronomic results. PVS was used in order to select new lines more suitable for this specific environment.

**Participatory Plant Breeding.** While in PVS the choice of farmers is restricted to the selection of varieties already existing, in PPB programs the farmers are taking part themselves to the creation of new varieties better responding to their specific environment and/or processing purposes. PPB trials are conducted within the SOLIBAM project and the farmers are assisted by the staff of the Rural Seeds Network. The experimental plots are sown with heterogeneous populations of wheat bread, obtained by the Italian scientist Salvatore Ceccarelli from all the possible crosses among 7 parental lines of a Hungarian wheat population, and the crosses among 21 parental lines of an English population. The result is called “Composite Cross Population” and presents the higher level of genetic diversity, which can be achieved from the initial parental lines. Wheat is autogamous, but in such heterogeneous populations the percentage of heterogamous pollination is expected to be higher

<sup>7</sup> The “price squeeze” indicates a situation in which the costs of production raise, while the prices of agricultural products do not increase in parallel, provoking a reduction of incomes.

than the average, with positive effects on biodiversity. When these population are re-sown year after year, in locations characterised by abiotic and/or biotic stresses, the increase of the frequency of the most adapted genotypes leads to a gradual adaptation to the local conditions of the environment - especially to those stresses they are facing more frequently, e.g. tardy frost, excess of rain, etc. (Ceccarelli, 2009). The high level of heterogeneity of these populations is beneficial especially in OA; in fact AE studies have demonstrated that agro-ecosystems can achieve a high degree of stability and resilience through biodiversity (Altieri and Nicholls, 2000). Within the SOLIBAM project, the same populations have been sown in 10 different farms across Europe to analyse their adaptive response to different environments and agronomic practices.

### **Theoretical background: The open innovation paradigm**

PPB can be considered as an innovative approach in organic seed selection processes. Hereby innovation is defined as new ideas, successfully implemented in organizational processes and outcomes (Dodgson and Gann, 2010). This may seem a strange claim, as seed selection appears to be part of natural evolutionary growth and development path within biology and agriculture, but when we compare this approach with the standard plant breeding methodology of private enterprises, we can see that these companies apply *closed innovation models* in their seed breeding processes. These models are based on a fading innovation paradigm of *science push*, where new ideas are created and applied in a closed environment of inbound research & development laboratories. The created intellectual property and associated revenues are protected through patenting of new varieties. This approach does not only make farmers more dependent on their suppliers in the food supply chain, but also suppresses innovation<sup>8</sup>. Kanter (2006) explains that the belief that innovative ideas can only be created in closed laboratories is a structural mistake in innovation management. New ideas, such as PPB, cut across different knowledge ‘silos’, sectors, and actors of the organic cereal value chain. Therefore PPB can be identified as an *open innovation model* in seed selection processes. Open innovation has been coined and defined by Chesbrough (2003), as meaningful search and dissemination of knowledge in order to speed up internal innovation processes, and enlarge opportunities for external use of innovation. Open innovation paradigm allows access to information by all the members of PPB projects and other actors interested in this breeding process. The process stresses absorptive capacity of data and knowledge by farmers, food processors, seed producers and suppliers, and promotes exploitation of external information. PPB endorses liberation of expertise and experience for other members of both the organic and conventional cereal supply chains. This is the same approach of AE, aimed at valorising different sources of knowledge, its sharing and appropriation. AE recognizes the importance of participatory approach, because even in the more advanced forms of agriculture, farmer’s knowledge has been recognized as a fundamental resource to enhance innovation (Ashby and Lilja, 2004).

**Social signaling value.** The open innovation paradigm and its implementation in PPB example should be seen as what Von Hippel (2006) calls ongoing democratization of innovation. “*Democratization of the opportunity to create is important beyond giving more users the ability to make exactly right products for themselves. (...) the joy and the learning associated with creativity and membership in creative communities are also important, and these experiences too are made more widely available as innovation is democratized*” (von Hippel, 2006, p.124). Therefore PPB has an important social signaling value in transforming the current food system through opening up the seed selection processes. The open model is responsible for new relationship between knowledge and economic value creation (Van Berlo and Jansen, 2013). This goes beyond the scope of sole seed production, as we are dealing with such topics as biodiversity and vivid ecosystems,

<sup>8</sup> “Closed innovation embraces a strategy of vertical integration and exclusive control” (Chesbrough, 2003, p.12).

livelihoods of farmers, urban-rural networks, and creation of learning and innovation networks for sustainable agriculture<sup>9</sup>.

**New organizational structure.** The open innovation model implies new ways of organizing the work processes in networks, clusters and even initiating co-creation among the cereal supply chain. Farmers can be seen as the ‘prime species’ within the agro-ecosystem; through the price squeeze this system gets perturbed and innovation initiatives stifled. The existing closed model leaves little space for innovation outside the R&D laboratories, within the current seed selection methodology. As a result, farmers together with other stakeholders react trying to create alternative food networks and innovation ecosystems based on agroecological values, where the partners of PPB projects act as custodians of biodiversity and local knowledge. These alternative food networks (AFNs) depend on their embeddedness into broader local social networks; they can renew the lost connection with producers, nature, land and the consumers because they not only add innovations into the food supply chain, but they also reintroduce the lost social component to the current food system, such as the need for cooperation, resilience, reduction of alienation, valuable relations and solidarity that represent social capital, trust, and increased adoptive capacity. The AFNs based on these values also tend to approach the environmental and economic aspects of the food system in a different way, which is expressed through their organizational structure into open innovation networks.

**Open for consumers.** Evolution of the open innovation concept will eventually lead to a bigger influence and involvement of concerned consumers. Examples of this future trend are the already existing Solidarity-based Purchase Groups (GAS<sup>10</sup>) in Italy and in particular in Tuscany region. According to von Hippel’s classification, these consumers groups can be considered as lead users of sustainable agricultural output and producer – consumer relations (Brunori, Rossi and Malandrini, 2011). *“That is, they are ahead of the majority in their populations with respect to an important market trend, and they expect to gain relatively high benefits from a solution to the needs they have encountered there”* (von Hippel, 2013, p.5). As Brunori, Rossi and Guidi (2012) have shown, GAS have the capacity to co-create together with other actors/members of food supply chains and food networks; the new way of co-production can lead to a more general change in the way innovation is applied in the seed selection processes, both within closed as well as open models. AE and PPB can add diversity to the existing production methods, and therefore challenge and increase its innovative capacity.

### Discussion and conclusions

The recent emergence and diffusion of PPB movement is considered as a response to the weaknesses of the conventional approach to plant breeding. Decentralized plant breeding approaches based on participatory methods offer the following advantages: i) improved local adaptation; ii) promotion of genetic diversity; iii) increased breeding efficiency and, iv) empowerment of local communities. PPB programs allow farmers to take part in the development of new varieties or populations, more suitable to marginal environments and to organic farming agronomic practices. The cereals obtained from such breeding methods can be successfully valorized through artisanal processing (bread, bakery, or pasta making), which is more flexible than industrial one and thus can be adjusted according to the characteristics of the flours (Morris and

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<sup>9</sup> LINSAs are defined as networks of producers, users, experts, civil society organizations, local administrations, formal Agricultural Knowledge System components, SMEs that create mutual engagement around sustainability goals in agriculture and rural development, and to this purpose they co-produce new knowledge by creating conditions for communication, share resources and co-operate on common initiatives (Moschitz, 2012).

<sup>10</sup> GAS - *Gruppi di Acquisto Solidale* - are Italian food networks run by concerned consumers and based on solidarity purchasing activities (Brunori et al., 2011).

Bellon, 2004). Participation is an important issue that still need to be implemented, especially the consumers' one, in order to be able to take into account also the needs of the end-users in the breeding process. At Pratini farm, the ongoing innovative participatory methods for organic cereal breeding, started and developed thanks to the fundamental interaction among the farmers and other partners: the Rural Seeds Network, the Tuscan Coordination of Organic Producers, the Universities of Florence and Pisa and the SOLIBAM European project. This participatory process led the farmers involved to a change in their approach to agriculture, which shifted from the closed innovation models typical of the 'Productionist paradigm' (based on intensification of agriculture through technological advancement) to open innovation models, typical of AE and the 'Ecologically integrated paradigm' (based on a more holistic management approach towards agroecosystems).

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## INFLUENCE OF FOLIAR APPLICATION OF GUANO ON GRAIN YIELD OF DIFFERENT CORN HYBRIDS IN ORGANIC PRODUCTION

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### Abstract

Studies were performed during 2011 and 2012 on experimental field of the Institute for Vegetable and Field Crops, In Department for Organic production and biodiversity in Ba ki Petrovac. The experiment was set up on certified plot, as two-factorial according to split plot design with four corn hybrids: ZP 555su, NS 620k, NS 609b and NS 6030 and two fertilization systems: foliar fertilization with organic fertilizer guano with increasing concentrations and treatment of seed corn variety by *Azotobacter chroococcum* strain also with three different concentrations. The aim of the study was to determine whether different concentrations of *Azotobacter chroococcum* strain with which the corn seed was treated and increasing concentrations of organic fertilizer guano, foliar applied through two fertilizations, affect the yield and quality of corn grain.

The results of two years lasting studies of joint action of foliar application of increasing guano concentrations and decreasing concentration of *Azotobacter* strain in hybrids NS 620k and hybrid NS-6030, significantly increased corn grain yield in comparison to the unfertilized control variant. In the treatment with the highest applied concentration of guano in fertilization, and the lowest concentration of *Azotobacter chroococcum* for popping corn seed treatment, statistically higher yield was achieved in comparison to the treatments with lower concentrations of the applied fertilizer and bacterium strain. Statistically, higher yield of hybrid NS 620k was achieved in 2011, at all treatments with joint action of guano and *Azotobacter*, in relation to the treatments only by *Azotobacter chroococcum*.

**Key words:** organic farming, guano, *Azotobacter chroococcum*, corn yield.

### Introduction

Production of organic corn in Serbia started recently and it is grown in small areas. However, number of organic food producers in Serbia keeps increasing due to growing market demand for healthy food ( Uvardi et al., 2006). Corn is primarily used as cattle feed, such as grain or silage, as raw material for industrial processing, but also as vegetables, seasoning, indispensable in many products of national cuisine, for making salads, popcorn, etc. Low in calories, and on the other hand with a lot of useful substances such as proteins, vitamin A, followed by group B, C and E, minerals Ca, Mg, Zn, Mn, beta carotene, starch, unsaturated fatty acids, etc. Basic principles of organic production systems include use of only natural inputs, i.e. application of cultural practices that preserve natural resources and enrich biodiversity (Bekavac, 2012). Corn has a high demands toward soil, nutrients and water, and therefore pronounced negative impact on resources and agroecosystems. In organic production system, use of more different sources of nutritive elements such as: soil organic matter, organic and natural fertilizers, cultivation of legumes and ploughing and/or composting of crop residues, application of biofertilizers is recommended. Increase in organic matter content in soil is a key precondition of organic production, as organic matter is the most important source of plant nutrients. However, very small amount of nutrients from organic matter is available to plants (Steen, 1994). General rule is that soils with a higher content of organic

matter have also higher number of microorganisms, and presence of microbiological communities depends on organic matter chemical composition (Bending & Rayns 2000). For this reason, use of organic fertilizers and some P and K fertilizers is allowed in organic production. Positive effect of fertilizers application is in increase of soil fertility and biological activity (Bogdanovi et al. 1995; Bo Liu et al. 2007). Microbiological fertilizers – biofertilizers contain chosen cultures of microorganisms and are used for inoculation of seed and nursery plants, or are incorporated into the soil in order to intensify certain microbiological processes that increase content of available plant nutrients (Jarak and uric 2008). Azotobacter fixes elemental nitrogen and produces biologically active matters and group B vitamins. Biologically active matters have beneficial effect on germination, growth of seedlings, plant growth and development (Mrkova ki & Mezei 2003). Therefore, the aim of the study was to determine whether combined action of Azotobacter chroococcum strain –with which seed of three corn hybrids was inoculated and liquid organic fertilizer guano, with foliar application through two fertilizations, as the source of nitrogen, affect the yield and quality of corn grain in organic production.

### Materials and methods

Studies were carried out during 2011 and 2012 on experimental field of the Institute for Vegetable and Field Crops. The experiment was set up on certified plot for organic production, as two-factorial according to split plot design in four replications. The experiment included four corn hybrids: NS 620k; NS 609b; NS 6030 ; ZP 555 šu, (in the paper are presented results for the first three hybrids) and two fertilization systems: the first system is application of biofertilizers – corn seed treatment by Azotobacter chroococcum strain, and the second system is foliar application of organic fertilizer guano (7:3:5) in two fertilizations.

Fertilization treatments:

Unfertilized - (control )

A1 + FG1 (Azotobacter chroococcum  $1 \times 10^8$  + 2% guano solution)

A2+ FG2 (Azotobacter chroococcum  $1 \times 10^6$  + 4% guano solution)

A3+ FG3 (Azotobacter chroococcum  $1 \times 10^4$  + 6% guano solution)

Before setting up of the experiment, soil samples for agrochemical analysis were taken from the layers of 0-3 and 30-60 cm (Table1).

Table1. Agrochemical soil properties before setting up experiment

Godina	Dubina cm	pH		% CaCO <sub>3</sub>	% Humus	% N	N min. (0-120cm)	mg 100g <sup>-1</sup>	
		H <sub>2</sub> O	KCl					P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
2011	0-30	8,00	7,17	5,44	2,87	0,14	71,87	18,94	22,93
	30-60	8,07	7,30	9,62	2,37	0,12		9,76	19,80
2012	0-30	8,06	7,19	4,62	2,63	0,13	42,89	22,27	22,66
	30-60	8,26	7,39	14,70	2,00	0,11		6,77	17,19

According to Table 1, soil in the experiment with corn is neutral to alkaline, medium to highly calcareous, in the middle class provided by humus and total nitrogen, with easily available phosphorus well provided in the first layer and in the second poorly, while in easily available potassium belongs to the class of good to medium provided soil. For agrochemical soil analysis standard methods were used.

For the purpose of monitoring dynamics and distribution of NO<sub>3</sub> – N per profile, soil samples were taken: before sowing, in the phase of tasseling and before corn harvest per layers of 0-30; 30-60; 60-90; and 90-120 cm. NO<sub>3</sub>- N was determined by N min. method of Scharpft and Wehrmann (1978). Grain yield, cobs per repetition and moisture were measured. Statistical data analysis

(ANOVA) was accomplished using the software GenStat Release 9.1 (Rothamsted Experiment Station, Trial Version).

Weather conditions in the analyzed years (according Latkovic 2013) indicate that the two years were extremely dry, in growing season 2011 fell only 212 mm of rainfall, i.e. for 148 mm less than LTA (360 mm). Also, in 2012, in comparison to LTA in growing season fell 130 mm less rain. However, winter moisture supplies (October – March) in 2011 were somewhat more convenient (266 mm), compared to 2012 (only 200 mm; i.e. 55 mm less in relation to LTA values). Thermal conditions in the analyzed years also did not support corn cultivation. In both years the average temperature values for growing seasons were higher in comparison to LTA, which was particularly pronounced in 2012 in three critical summer months (June, July, August) when the average monthly air temperatures significantly exceeded LTA values

### Results and Discussion

In Table 2 are presented yields of three corn hybrids per fertilization treatments in studied years. Results of F-test show that in 2011 there existed statistically highly significant difference in grain yield between the studied hybrids. In 2012 between grain yield of the hybrid NS 620k and other two hybrids existed statistically significant difference, while it did not exist between grain yield of the hybrids NS 609b and NS 6030. The highest average grain yield in both years had hybrid NS 6030, and the lowest NS620k. Significantly higher grain yield achieved for all three hybrids in 2011 in comparison to 2012 can be explained by favourable climatic conditions, larger reserve of the available winter moisture and lower temperatures in 2011 growing (Latkovi et al., 2013).

Table 2. Yield of corn hybrids as dependent on fertilization treatments in 2011 and 2012.

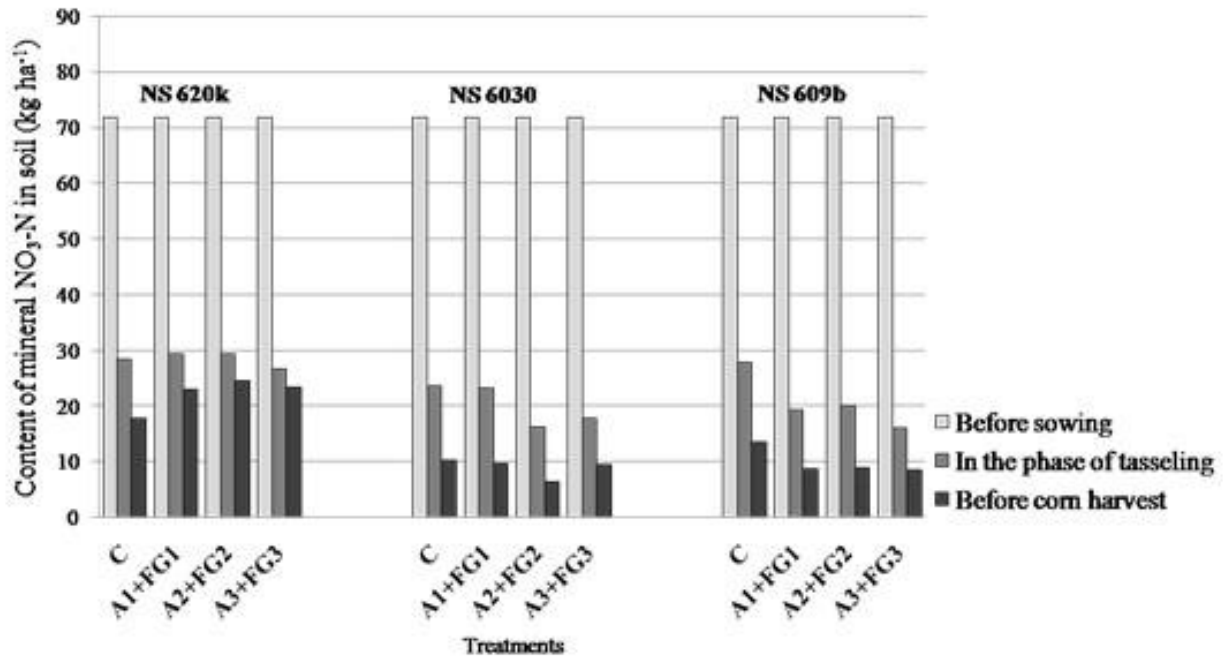
Hybrids Treatments (T)	NS 620k (H) t ha <sup>-1</sup>		NS 6030 (H) t ha <sup>-1</sup>		NS 609b (H) t ha <sup>-1</sup>	
	Year (Y)		Year (Y)		Year (Y)	
	2011	2012	2011	2012	2011	2012
<b>C</b>	4,403	2,378	10,656	5,199	8,746	5,009
<b>A1</b>	4,971	1,781	10,901	5,492	8,591	5,470
<b>A1 + FG1</b>	4,425	2,749	10,322	6,425	9,056	4,924
<b>A2</b>	4,887	1,762	10,753	6,900	8,866	5,811
<b>A2 + FG2</b>	4,288	2,135	10,615	6,283	8,969	5,549
<b>A3</b>	4,301	4,301	10,956	6,397	8,925	5,137
<b>A3 + FG3</b>	4,730	4,730	10,807	6,063	9,041	5,339
	<b>LSD</b>	Treatments 2011	Hybrid 2011	Treatments 2012	Hybrid 2012	
	0,05	0,4973	0,4553	0,7821	0,8668	
	0,01	0,661	0,6898	1,0395	1,3132	

In comparison to highly significant differences in grain yield between various hybrids, the applied fertilization treatments had lower impact to the yield. In 2012, for hybrid NS 620k treatments A3 and A3+FG3 resulted in significantly higher grain yields in relation to all other fertilization treatments and control. Grain yield of the hybrid NS 6030 in 2012 in all treatments with foliar application of guano and Azotobacter was statistically higher in comparison to the treatment with Azotobacter alone in the lowest concentration (A3) and control – without fertilization. On the other hand, in 2011 for the same hybrid significant difference in grain yield was established only between treatment A1 in comparison to the treatment A1+FG1. In 2012 the same hybrid had significantly higher yield at treatment A1 compared to treatment A3 and control.

On Graph. 1 and 2 dynamics of NO<sub>3</sub>-N in the soil is presented: before sowing, in the phase of tasseling and before harvest of the corn hybrid. In organic production corn plants absorb mineral



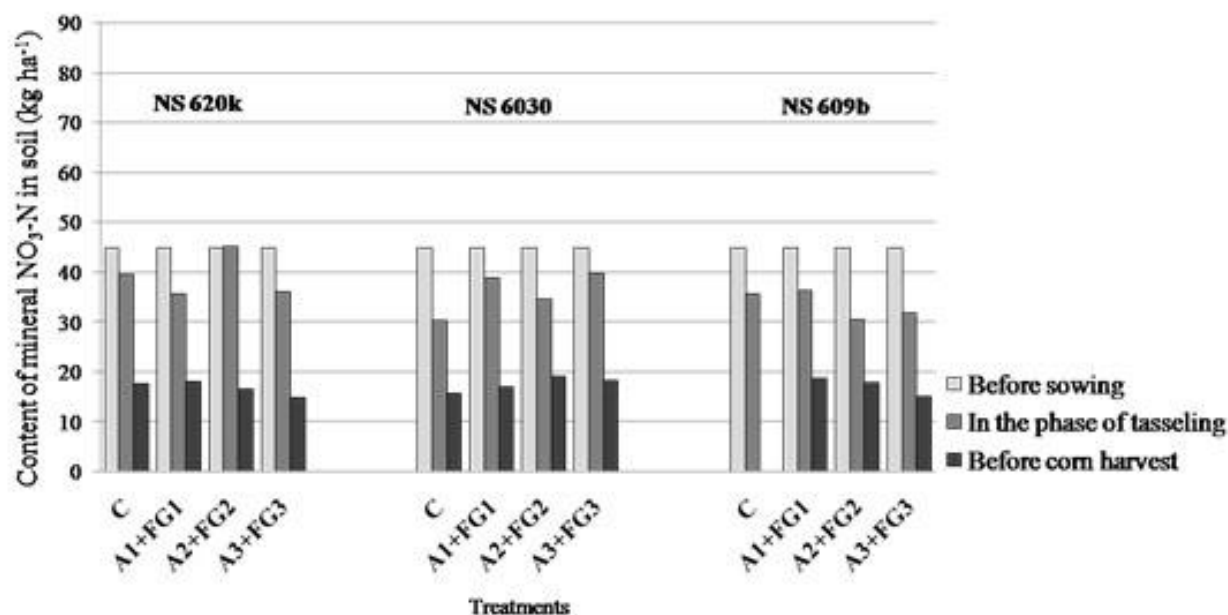
nitrogen from the soil produced by mineralization of organic matter, while in conventional production in addition to mineral nitrogen from organic matter (depending upon soil fertility), plants absorb also nitrogen from the applied mineral N-fertilizers (Bogdanovi et al., 2010). In both years of the study before sowing of corn hybrid according to the measured quantities of  $\text{NO}_3\text{-N}$ , the soil was in the class of good assurance (Graph 1 and 2), although the experiment was performed on the plot that was for many years used in organic farming – without use of mineral N-fertilizers (every four years it is fertilized by manure).



Legend for Graph 1. and Graph 2: C - unfertilized control; A1 FG1 - Azotobacter chroococcum  $1 \times 10^8$  + guano solution 2%; A2 FG2 - Azotobacter chroococcum  $1 \times 10^6$  + guano solution 4%; A3 FG<sup>3</sup> - Azotobacter chroococcum  $1 \times 10^4$  + guano solution 6%

Graph 1.  $\text{NO}_3\text{-N}$  dynamics in the soil under different corn hybrids in 2011.

Good assurance of the soil by  $\text{NO}_3\text{-N}$  before sowing of the corn hybrid can be explained by mineralization of organic matter of the soil (soil humus content in the experiment was between 2.63 and 2.87%), crop residues and roots of previous crop and microbial biomass (Bogdanovi and Ubavi 2008; Manojlovi et al. 2008). In these studies, treatments of corn seed by different concentrations of Azotobacter chroococcum strain were performed in order to supply corn crop by mineral nitrogen over fixation of elemental nitrogen, with two foliar applications of organic fertilizer guano during growing season. Studies of Govedarica et al. (2001) suggest that use of certain bacteria strains in conventional corn production can lower quantities of N-fertilizers, and thus lower costs of production. Use of biofertilizers in corn production provides cheaper and ecologically highly valuable food (Hajnal and Govedarica 2004). According to the studies Hajnal and Govedarica (2004) microorganisms - diazotrophs have positive effect on seed germination, growth of seedlings, growth and development of plants and also on the yield and its quality not only by fixing elemental nitrogen, but also by producing some other biologically active substances (such as hormones and vitamins). Monitoring of  $\text{NO}_3\text{-N}$  dynamics in the soil under experiment, from planting to harvest of corn hybrids, per dates of sampling, revealed that its quantities reduce in view of the crop demands for nitrogen during growing season and depending on the hybrid.



Graph 2. NO<sub>3</sub>-N dynamics in the soil under different corn hybrids in 2012.

### Conclusion

Based on results of two years researches in field experiments with three hybrids the following can be concluded:

In experiments carried out in 2011, all three hybrids in all fertilization treatments had statistically significantly higher yield in comparison to grain yield in 2012.

In 2011 statistically significant difference was established between grain yields for all three corn hybrids.

In 2012 significant difference was established between grain yield of the hybrid NS 620k and the other two hybrids, while between hybrids NS 6030 and NS 609b significant difference did not exist. Influence of fertilization systems: treatment of the hybrid corn seed by *Azotobacter chroococcum* strain and foliar fertilization by different concentrations of organic fertilizer guano had significantly lower effect to grain yield in relation to the effect of hybrids in both years of the study.

In 2012 all three guano concentrations for fertilization of the hybrid NS 6030 in comparison to control showed statistically significant effect to the grain yield.

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## EFFECT OF MICROBIOLOGICAL FERTILIZERS AND ZEOLITE ON YIELD OF WINTER RYE UNDER HIGH ALTITUDE CONDITION

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### Abstract

Effect of microbiological fertilizers (Uniker and Slavol) and soil additive, zeolite on winter rye yield was investigated in this paper. Trial was set up in Akmadžici village in agroecological conditions of Zlatar mountain on altitude of 1065m, during two-year period 2010/11 and 2011/12. A randomized complete block design with three replications was employed. In organic cropping system three variants of microbiological fertilizer with zeolite were used prior to sowing (Zeolite, Uniker and Zeolite+Uniker). Half of each plot was treated with foliar microbiological fertilizer Slavol during crop growing period.

On the basis of two-year results it is obvious that meteorological conditions have very significant influence on winter rye yield. The big difference between temperature and precipitation has effect on significant differences between yield in two seasons. The second season, 2011/12 had weather pattern less favorable for the rye production due to severe drought. Different combinations of the microbiological fertilizers and the soil additive gave positive results specially in the first year of the trial. The best combination in organic cropping system was Uniker + Zeolite with foliar application of microbiological fertilizer Slavol, which resulted with the greatest yield of winter rye and this treatment can be recommended to producers. Winter rye performed very well under limited conditions of acidic soil on high altitude in organic cropping system and it can be recommended as very suitable crop for organic producers.

**Key words:** winter rye, microbiological fertilizer, soil additive, organic cropping system, grain yield.

### Introduction

Winter rye is very cold tolerant and is the hardiest and most disease resistant of the winter cereals. Winter rye is earlier and faster growing in the spring than the other winter cereals, including wheat and barley (Malešević et al, 2008). It is an excellent source of organic matter, grows in marginal soils, reduces erosion and has an allelopathic action that suppresses weeds (Teasdale et al, 1991). Rye is one of the best cool season cover crops for outcompeting weeds. If climatic conditions favor grain development, it will produce a better crop than any other grain on infertile, poorly tilled soil, tolerates very acid soil conditions (Glamo lija, 2004). Therefore, rye is very suitable for growing in organic cropping system on high altitude plots (Olja a et al. 2010).

Rye's nutritional characteristics are similar to the other cereal grains, however rye is higher than wheat in fiber, vitamin E, riboflavin, folacin and pantothenic acid. Unusual for a cereal grain, rye contains twice as much of the amino acid, lysine as wheat. This is especially significant because lysine's the limiting amino acid in wheat and most other cereal grains which necessitates food mixing to develop a complete protein. This isn't a problem with rye as eating rye by itself gives you a well rounded protein (Diousse and Gaziano, 2007). Rye contains a lot of soluble fibre which slows down the release of carbohydrates and sugars, so that you feel satisfied for longer after eating it compared to wheat bread. The sugars in rye are largely "fructans" - a type of fructose, which accounts for the slightly sweet taste. Fructans allow this plant to thrive in relatively cool conditions. Many of the benefits of eating rye come from the fact that it ferments in the gut to produce valuable

nutrients such as short-chain fatty acids and arabinoxylan. Short-chain fatty acids help the immune system by promoting lymphocyte production and they also lower cholesterol production and stabilise blood sugar levels. Arabinoxylan is thought to act much like beta-glucan from oats. Beta-glucans are responsible for some of the heart-healthy attributes of oats and have a whole bundle of health benefits credited to them (Truswell, 2002).

### Material and methods

Rye cultivar Raša were included in a complete randomized block design with three replications with sowing rate of 200 kg ha<sup>-1</sup>. This cultivar created in Center for small grains in Kragujevac, has very good tolerance on cold temperature, drought and the most diseases (Milovanovic et al. 2005). The grain is slightly bigger than other cultivars and protein content is high 15,8 %. Trials were set up in Akmadžici village (43° 25' 27" N, 19° 52' 13" E) under agroecological conditions of hilly-mountainous region of Zlatar (altitude 1043 m) during two-season period 2010/11 and 2011/12. Sowing was performed on October 05<sup>th</sup> and October 10<sup>th</sup> for growing seasons of 2010/11 and 2011/12 respectively, and harvesting on July 27<sup>th</sup> and 31<sup>th</sup> respectively. Weather data covering three seasons were collected from the nearest meteorological station Zlatibor (Figure 1). The season 2010/11 was characterized by higher mean temperature (7,2°C) then in 2011/12 (6,8°C). The lowest monthly mean temperature was recorded in February 2012 (-7,3°C). Precipitation sum during vegetative period in 2010/11 was 882 mm and in 2011/12 it was 706 mm, which is lower than long-term average.

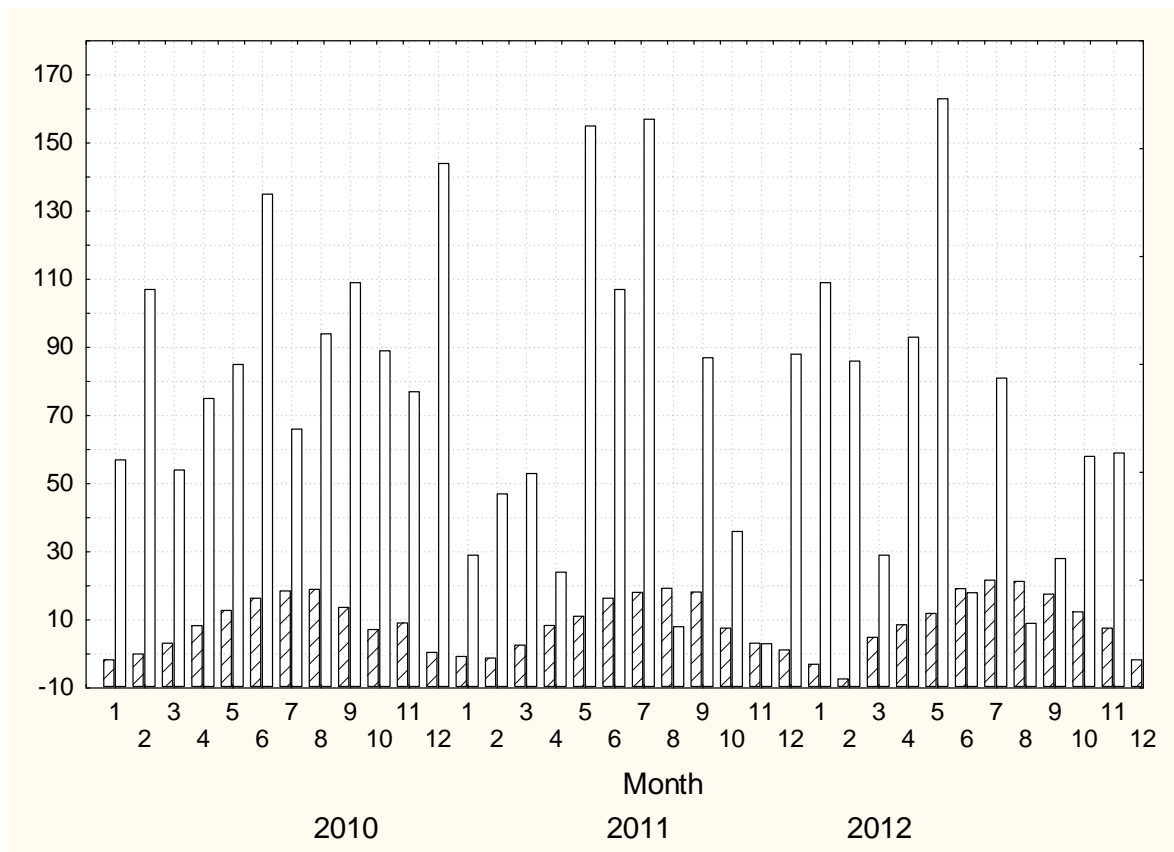


Figure 1. Meteorological conditions for Zlatibor in period 2010- 201

A plot size was 12m<sup>2</sup> and the soil was very acid with high content of humus, high reserve of mineral N, and very low content of P (Table 1). A randomized complete block design with three replications was employed. Soil additive zeolite (2,67 t ha<sup>-1</sup>) and microbiological fertilizer Uniker (10 t ha<sup>-1</sup>) and their combination were applied on the top soil prior to sowing. In organic cropping

system three variants of microbiological fertilizer with zeolite were used (Zeolite, Uniker and Zeolite+Uniker). Half of each plot was treated with foliar microbiological fertilizer Slavol during crop growing period in amount of 7 l ha<sup>-1</sup>. The microbiological fertilizers Uniker and Slavol are universal, certified fertilizers and can be used in organic agriculture. These are natural products without any chemical additives, thus having a positive influence on plants, soil and the environment. The soil additives zeolite is also allowed for application in organic agriculture. Zeolite can act as water moderators, in which they will absorb up to 55% of their weight in water and slowly release it under the plant's demand. This property can prevent root rot and moderate drought cycles.

Grain yield of winter rye (t ha<sup>-1</sup>) were determined after harvest. The data were processed by ANOVA, using LSD test for comparison of means.

Table 1. Soil properties on experimental plots

Depth (cm)	pH		CaCO <sub>3</sub>	Humus	Total N	C/N	Available N			Available (Al-method)	
	H <sub>2</sub> O	nKCl					%			NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>
			mg/kg				mg/100g				
0-10	5,00	4,00	-	5,32	0,297	10,6:1	17,3	17,6	34,9	1,1	15,1
20-40	4,63	4,07	-	4,04	0,210		18,1	12,2	30,3	0,5	9,6

## Results and discussion

On the basis of two-year results it is obvious that meteorological conditions have very significant influence on the rye productivity. The first season, 2010/11 had weather pattern more favorable for the rye production with mild and moist winter and warm but rainy spring and summer. The big difference between temperature had effect on significant differences between yield in the two seasons. Significantly greater rye yield was recorded in 2010/11, because of very low temperature in winter 2011/12 and lack of precipitation in early summer 2012 (Table 2).

Different combinations of the microbiological fertilizers and zeolite gave positive results on grain yield compared with the control. The best combination in two-year average in organic cropping system was Uniker + Zeolite with foliar application of the microbiological fertilizer, which resulted with the greatest yield of winter rye (2.07 t ha<sup>-1</sup>). It is obvious that foliar application of the microbiological fertilizer has very positive influence on rye productivity except in variant with Uniker. In variant of the control with no fertilizers but with foliar application of the microbiological fertilizer we obtain approximately the same result. This result is very much compatible with the results of Kova evi et al (2009, 2011) where significantly greater yields of different species of wheat (*Triticum spelta*, *T. durum*, *T. aestivum ssp. compactum*) and other small grains were obtained in similar treatments. It is also in accordance to the results of experiment with winter rye (Olja a et al, 2010) in organic cropping system under agroecological conditions of Valjevo region.

The highest yield improvement that has been achieved by application of the microbiological fertilizer was in the variant Uniker + Zeolite + Slavol fol. in 2010/11 with 2.14 t ha<sup>-1</sup> and this combination can be recommended to producers (Table 2). Yield in the control was not significantly lower in growing season 2010/11 compared with other treatments thanks to high reserve of nitrogen and humus in the soil. It is important to stress that soil on experimental plots was not used for agriculture for a long period of time, retain fertility and enabled greater crop productivity. Natural microbiological fertilizers such as Uniker and Slavol containing products of bacterial fermentation, natural vitamins, enzymes and growth stimulators helps the plant to more effectively bind useful substances from the soil, exploiting natural resources to the maximum, without polluting the soil. They aid the nutrition of plants by converting organic and hardly soluble compounds into accessible

forms which are directly delivered to the root systems. For organic producers, especially in neglected hilly-mountainous regions of Serbia, these results might be helpful.

Table 2. The effect of microbiological fertilizers and zeolite on yield of winter rye

Variant	Yield (t ha <sup>-1</sup> )		Average
	2010/11	2011/12	
Control	1.65	1.52	1.59
Control + Slavol fol. Zeolite	1.78	1.51	1.65
Zeolite	1.69	1.58	1.64
Zeolite + Slavol fol.	1.87	1.97	1.92
Uniker + Zeolite	1.95	1.90	1.93
Uniker + Zeolite + Slavol fol.	2.14	1.99	2.07
Uniker	1.82	1.72	1.77
Uniker + Slavol fol.	1.87	1.58	1.73
Average	1.85	1.72	

LSD 0,05	LSD 0,01		A-year, B-foiliar fertilizer, C-fertilizer
0,0466	0,0665	A	
0,0680	0,1163	B	
0,0785	0,1343	C	
0,1111	0,1899	AC	
0,0962	0,1645	AB	
0,1360	0,2326	BC	
0,2221	0,3799	ABC	

### Conclusions

According to the presented results of the research of different microbiological fertilizer combinations with soil additive zeolite in organic cropping system for winter rye during the seasons 2010/11 and 2011/12 following conclusions can be stated:

The first season, 2010/11 had weather pattern more favorable for the winter rye production. Organic cropping system under conditions of Zlatar hilly region gave significantly greater yield compared with the control specially in 2010/11 growing season. The best combination in two-year average in organic cropping system was Uniker + Zeolite with foliar application of the microbiological fertilizer Slavol which resulted with the greatest yield of rye. It is obvious that foliar application of the microbiological fertilizer has very positive influence on crop productivity in both years of the trial except variant with Uniker, which leads toward conclusion that this treatment can be recommended not only for rye but the other alternative small grains. On the other hand winter rye performed very well under limited conditions of acid soil on high altitude in organic cropping system and it can be recommended as very suitable crop for organic producers.

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**AGROSYSTEMS PROTECTION AS HERITAGE ELEMENTS:  
CULTURAL LANDSCAPES**

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**Abstract**

Transformations in agriculture during the last decades have generated a type of industrial intensive farming based on big inputs of energy and agrochemicals, which is considered a major cause of environmental imbalances. Moreover, historical cultures are generally sustainable, provide biological and landscape diversity, and often beauty. Frequently they form valuable heritage spaces. The human need for subsistence food production has kept them alive to this day in many places around the world.

Traditional crops are not natural: they need constant human action for maintenance. But the negative impact in the environment is much lower than in industrial agriculture. Many traditional agrosystems are properly Cultural Landscapes. These areas are being abandoned widespread in developed countries, and simultaneously with the spaces also the traditional social relations system. There are legal protection figures from heritage perspective, as UNESCO World Heritage, Landscape European Convention, or local legislation that can help in the aim to safeguard these ancient places.

We studied the Palmeral de Elche (Spain). It constitutes a representative example of this typology, which has been included in the World Heritage List. There we had a prospect over general aspects of degradation in a present urban transformed environment. It is a major item for conservation to understand his significance and strategic value. We are not only losing elements of the agrosystem, but also the ancestral culture associated with its maintenance and operation. We afford some ideas to evaluate landscape values and to understand essential nuances and places to preserve.

**Keywords:** Agrosystem protection, Sustainability, Cultural Landscape, World Heritage, Palmeral de Elche

**Overview**

World agriculture undergoes a series of transformations caused by the evolution of the global economy, in a more intense way since the last decades of the twentieth century. The situation is evolving into a kind of industrial intensive farming, in huge farms, maintained on the basis of strong energy inputs, machinery, seeds and chemicals, and is considered a major cause of environmental and social imbalances. The change from family or subsistence agriculture to an industrial one is a process common to all developed countries.

Moreover, historical farming is often sustainable, provide biological and landscape diversity and often beauty. Agricultural productivity measured in kg/ha, and economic performance, are lower than in industrial crops. But these are not the only items to be considered. There is also a smaller plot size, the minimum landscape tile. As a result, the extension of the separation lines is much larger in family plots and it represents a basic biodiversity redoubt. J. Lovelock (1993) uses the term "agribusiness vandals" and criticizes some environmentalists for its myopia regarding the disappearance of boundaries between small properties, which were the remains of the original natural matrix.

Traditional crops are not natural; they need human action for maintenance. The whole system falls apart quickly if it is left to their fate. They are designed or modulated from a low intensity use, so their overall impact on the environment is low. The relatively new concept of Cultural Landscape represents almost a new paradigm in planning, landscape or environmental studies. There are different criteria for recognizing a cultural landscape (<http://whc.unesco.org/en/culturallandscape/> Accessed on 07.15.2013).

Here we focus on traditional agricultural environments (Fig. 1,2,3). These areas are being abandoned or destroyed in developed countries (Küster, 2004). Many of them have heritage value in a broad sense of the word, particularly before to the risk of permanent loss. Understanding strategic significance of agrobiodiversity (agricultural and of livestock) conservation and food security is often poor. If we lose these spaces we not lose only elements from the territory, but also the culture accumulated over generations associated with maintenance and operation, usually in a sustainable manner.



Fig. 1. Grapevine Landscape. Vadobbiabene, Italy. From ([www.cultural-landscapes.ca](http://www.cultural-landscapes.ca))

Fig. 2. Terraces in Bali. ([weloveyatours.blogspot.com.es](http://weloveyatours.blogspot.com.es))

Fig. 3. Black Forest, Germany (autor Hans Page)

Cultural landscapes represent the "combined works of man and nature". They are illustrative of the evolution of society and human settlements along the years under the influence of the limitations and advantages of the natural environment, as well as the influence of the successive social, economic and cultural forces. Its area is defined by its functionality and intelligibility. The agro-cultural landscapes are being increasingly recognized as collective heritage items. The concept overlaps to a large extent with the category V established by the IUCN (Dudley N., Ed, 2008).

The early twenty-first century years requires a change of general attitude that involves the recognition of the securities and sustainability held by the traditional ranching and farming, as they are: maintenance of agro-biodiversity, food security, social cohesion, cultural values, etc. We must look ahead to the current reality to affirm the value of the traditional world against the simple logic of global currency markets. We must not simply accept the loss of assets, while it is necessary to redirect the ongoing processes with realistic conservation criteria, provided with the available means and resources.

There are adequate policy instruments and international conventions for the new reality, which reflect the plurality of confluent approaches and regulations and the most recent approaches to environmental heritage. It may be interesting to mention: the European Landscape Convention (<http://conventions.coe.int/Treaty/en/Treaties/Html/176.htm> Accessed on 07.14.2013), the UNESCO program for Biosphere Reserves, UNESCO World Heritage List (<http://whc.unesco.org/en/list/> Accessed on 15.07.2013), the FAO program Globally Important Agricultural Heritage Systems (<http://www.giahs.org/> Accessed on 16-07-2013), Natura 2000, based on the EU Habitats Directive, Rio and Rio + 20 agreements on biodiversity, the World Database of Protected Areas (<http://www.wdpa.org/> Accessed on 14-07-2013), in addition to regulation at national or local level.

In our experience is important to note that the attainment of an agricultural inclusion in any of these listings does not guarantee preservation, as we found in some points of the *Palmeral de Elche*. The effective achievement of this objective assumes the availability of human and material resources. It requires the existence of active and engaged local communities with efficient management mechanisms, in addition to technical and economic support.

UNESCO develops from the 70's one World Heritage List which contains over 700 properties, of which 80% are cultural, and the rest natural. Most cultural objects are buildings, monuments, archaeological sites and the like, but year by year the number of cultural landscapes in the List is increasing. Today there are some 80, many traditional agricultural areas. In 2000, the Palm Grove of Elche (Spain), an agrosystem cultural landscape was included in the List.

### Palmeral de Elche

UNESCO inscribed the *Palmeral* (palm grove) on the World Heritage List as a cultural element (<http://whc.unesco.org/en/list/930> Accessed on 14.07.2013) at the XXIV meeting of the World Heritage Committee in December 2000. UNESCO considered that it meets the requirements (ii) and (v) of the six available, in addition to authenticity, in the following terms:

- Criterion ii. The Palmeral (palm grove) of Elche represent a remarkable example of the transference of a characteristic landscape from one culture and continent to another, in this case from North Africa to Europe (Fig 4,5).



Fig. 4. Palmeral de Elche. Google maps      Fig. 5. The Palmeral from medium distance (1997)

- Criterion v. The palm grove or garden is a typical feature of the North African landscape which was brought to Europe during the Islamic occupation of much of the Iberian Peninsula and has survived to the present day. The ancient irrigation system, which is still functioning, is of special interest (Fig. 6,7).



Fig. 6. Torre de Vaíllos



Fig. 7. Traditional irrigation

The Palm Grove of Elche, according UNESCO, is an asset of outstanding universal value, representing agriculture and traditional uses. It is formed by a population of *Phoenix dactylifera* in the medieval city environment. It was created by humans as a kind of oasis, with an irrigation system on the basis, around the tenth century (Azuar, 1998, Martinez, 2001), with agricultural functionality. It is a part of the process that some authors (Glick, 1979) called "green revolution", associated with the spread of Islam in medieval Spain. Its foundation was simultaneous with the change of location of the city. According to this criterion, Palm Grove of Elche is the last oasis and the northernmost of those related to the expansion of Arab culture from the original focus on the Arabian Peninsula to its settlement in Spain (Sharq Al Andalus). It is the only large oasis in Europe.

The palm grove of Elche has an importance similar to other recognized elements of the Spanish-Arabic era. Its primary function was to produce food for the people in their immediate environment, as well as the creation of a microclimate suitable for this purpose. In Elche, with about 300 mm of annual precipitation, water for irrigation is scarce and saline. The role of the palm tree was relevant in this transformation, as far as palm tree is a salt resistant species, in addition to its high aesthetic and symbolic value in all the Mediterranean cultures. The Muslims who resettled the palm grove of Elche designed it as a tool to obtain maximum yields in harsh environmental conditions.

From the thirteenth century, with the Christian conquest, the city experienced a change of cultural influence, but many of the uses of the previous stage remained alive (Cavanilles 1795-1797). The palm grove was nestled in the heart of a culture different from his birth, which permeated in many aspects. Its strategic productive value was high at the time, and the degree of integration into the new dominant culture was such that allowed him to reach a functional state almost until today, going on through all kinds of circumstances and difficulties. UNESCO considers a key value the multiculturalism feature displayed in this environment. The palm grove has been for at least a thousand years an agricultural oasis whose function was food production and self-sufficiency, looking for basic materials at the local scale. The basic territorial unit is the huerto (Ortiz C., L. Gracia. 2000). Palm grove vocational activity is agriculture. The traditional irrigation system is still running, with changes, but UNESCO recognizes his value as a part of heritage.

The palm grove, as well as other cultural landscapes, represents a complex system of customs, relationships, social habits, associated rights over water, etc., characterized by sharing in scarcity, which seems to have passed from Muslim to Christian culture with little variations. Traditional production techniques are part of the intangible legacy and they should be object of worthy attention (Fig. 8, 9) UNESCO has recognized the character of Best Safeguarding Practices of the Intangible Cultural Heritage for the pedagogic project of the Center For Traditional Culture - School Museum of Puçol (Elche) (<http://www.museopusol.com/> Accessed on 14.07.2013), which has collected important elements of local culture, acting from a rural school with local community support.



Fig. 8. White palm



Fig. 9. Dates Biodiversity

The rapid socioeconomic changes and the availability of food and materials from remote sources have caused a breakdown in the ancestral system of palm grove exploitation. The functional traditional huerto is an increasingly rare figure (Fig. 10) and is a clear example of agrosystem which has lost his ancient function, because of the changes in social and productive model (Ortiz C. 2002).



Fig. 10. Urban uses in ancient *huertos* (2012)

There exist legal rules establishing different protection figures since 70 years ago, but the practical reality exceeds its regulatory capacity. Under our experience, the simple superposition of new statutory protection over those previously existing may be useless if suitable means are not developed, as well as clear procedures for his effective implementation. Despite legal protection, heritage threats have not disappeared. Usually it is caused by the pressure of the socioeconomic environment, and sometimes it is also due to the lack of understanding of the protected environment by a part of the population and by some of the managers. The transformations involve processes that may lead to a partial or total distortion of the agrosystem (loss of authenticity). If necessary, a system of reasonable and compatible new uses should be proposed for high-risk areas, after getting consensual agreements between stakeholders, along with a good management plan and efficient executive institutions. Decision making is a process of diagnosis and planning, targeting a well-defined set of objectives, which requires work and time to reach the scale of individual project. *State and Management Indicators* for the site are particularly useful even though developing them may be laborious. It is essential to enhance traditional palm farms under current heritage criteria (Gracia L. 2007).

The ideas that we present in the paragraphs above constitute only one of the first steps in the approximation to reality. In later phases of protection process will be essential to consider the overall intangible organization, and to know the environment to be protected at a detailed level, starting with the most valuable points. In our research we need to consider the economic aspects related to the management, in order to ensure its effectiveness. It is very difficult to maintain a farm

today as it was centuries ago, but we must not allow the erosion of the entire heritage. Cultural tourism, education and science are emerging basic references to consider today from UNESCO viewpoint. It is extremely important to recognize agrobiodiversity value, the relevance of local food security as well as the related social structure. They are values to be defended in a world dominated by economic globalization, with the involvement of local communities and institutions.

This is a small summary of our experience in the technical work of protection in a particular element of World Heritage. As we have pointed out, there are other legal or social protection tools, from local actions to other more extensive levels. Any of them will fail if there is no will, knowledge and means to implement it. Our primary goal in this contribution is to relate the world of traditional extensive agriculture with heritage assessment criteria, biodiversity conservation, sustainability, and some other applicable in each case. The value of historical agricultural systems is becoming clear for international institutions as elements of environmental and social stability, also as generators of important cultural expressions.

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**PRINCIPLES OF ORGANIC VITICULTURE APPLIED IN MURFATLAR VINEYARD,  
ROMANIA**

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**Abstract**

The paper describes the particularities of the organic viticulture technology applied in Murfatlar vineyard. The research was conducted during 2011 and 2012, for two romanian varieties: Columna, for white wines and Feteasca Neagra, for red wines. The main climatic indexes were determined: the real heliothermic index (IHr), the bioclimatic index (I.B.V.), the oenoclimatic aptitude index (IAOe) and the aridity index (Martonne), as well as the soil hydric regime and the dynamics of some physiological processes (stomatal conductance and chlorophyll index).

Due to the climate warming and water deficit, the phytosanitary status of plant were good but the timing of full maturity was advanced by 5 - 6 days in 2012 compared to 2011.

After evaluating the harvest quality, it was found that during the studied years the conditions were favorable for obtaining quality wines - DOC (about 200 g/l sugar and 6-7 g/l tartaric acid in grape must).

**Keywords:** Murfatlar vineyard, organic viticulture, adapted technology, harvest quality

**Introduction**

At the Research Station for Viticulture and Oenology Murfatlar vines are grown organically since 2007. The purpose of organic viticulture is to offer consumers a quality wine, typical for the region of origin. Organic Viticulture provides customers healthy products, without using chemical fertilizers, synthesized insecticides, fungicides and herbicides. One of the main goals of ecological agriculture is respecting the environment and therefore avoid the pollution of soil, air and water.

Currently, there are 45 ha of registered organically grown vineyards, of which 15 ha are certified and 30 ha under conversion. The varieties selected and cultivated under this system are Columna and Chardonnay for white wines and Feteasca Neagra, Cabernet Sauvignon and Pinot Noir for red wines.

Organic viticulture principles applied at Murfatlar is based on maximum use of local resources, in parallel to minimizing the economic and environmental risks (Bernaz, Dejeu, 1999). It aims mainly: maintaining the biodiversity, preserving the biological activity of the soil, preventive crop protection and obtaining quality productions.

**Material and methods**

The study was conducted at the Research Station for Viticulture and Oenology Murfatlar during the viticultural years 2011 and 2012. The observations targeted two organically cultivated vine varieties, Columna and Feteasca Neagra, focusing on the adaptability to organic cultivation in Murfatlar area conditions. The varieties under study were grafted the rootstock Berlandieri X

Riparia Teleki 4 - Oppenheim selection 4-4 and the training system used is Guyot with bilateral cordons.

Climatic data were provided by the weather station Weather Master 2000 and include daily observations regarding maximum and minimum temperature, insolation and precipitations, based on which were calculated a range of climate indicators frequently used in viticulture: heliothermic index, bioclimatic index, oenoclimatic aptitude index and aridity index (Martonne). In order to study the hydric regime of the soil, samples were collected for the depths of 0-20 cm, 20-40 cm, 40-60 cm, 60-80 cm and 80-100 cm for each of the 2 parcels under study. Sampling was performed using an agrochemical auger and the samples were processed by the gravimetric method (Blaga et al., 2005).

Weekly, between the phenological phases of blooming and ripening of the grapes measurements were performed directly in the field in order to determine stomatal conductance and chlorophyll index by using the SC-1 leaf porometer and SPAD-502DL chlorophyll meter.

Only phytosanitary treatments permitted by the legislation were used, namely: sprayings with copper sulphate - KOCIDE 2000 at a dose of 1.5 kg/ha and 80% sulfur - Kumulus DF, at a dose of 3 kg/ha. In order to monitor the moth populations (*Lobesia botrana*) were used sex pheromone traps.

In order to determine the production quality the grape ripening process was monitored, namely: the weight of 100 grains - by weighing with a technical balance, the must sugar content - refractometrically and the total acidity - by titration with 0.1 N NaOH with phenolphthalein as indicator.

The derived wines were analyzed by standardized physicochemical methods: the alcohol concentration - by the pycnometric method, the reducing sugars - by the Schrol method, the total acidity - by titration with 0.1 N NaOH in the presence of fenolroth as indicator, the volatile acidity - by distillation and titration with NaOH 0.1N in the presence of phenolphthalein and the unreduced extract - by the Tabarie method.

### Description of applied technologies

For the varieties under observation, before the vegetation period starting, were applied the following works: winter pruning, crop sanitizing and the revision of the sustaining system.

During the vegetation period were applied 4 hand hoeings, weeding sprouts on the stem, directing and tying the shoots, works that increased the efficiency of the applied treatments.

Soil tillage focused on preservation and raising fertility, water retention and accumulation, air penetration to the roots, the release of carbonic acid in the soil and facilitating the microflora activity. Five mechanical hoeings were performed for weed control. To protect vines against extreme temperatures and removing pests, autumn plowing was performed at a depth of 20-28 cm and spring plowing at a depth of 18-20 cm. It was taken into consideration the prevention of artificial soil compaction.

In order to optimize the ripening and maturation processes were applied the thinning-out, the shoot top and leaf removal, favoring production quality by increasing the concentration of sugars in wine.

## Results and discussion

### Climatic indexes

The real heliothermic index (IHr), proposed by J. Branas et al. (1946), emphasizes the interaction between light and temperature. The values obtained for 2011 and 2012 falls within the specific range for the Murfatlar vineyard, exceeding the value of 2.6, which indicates that the region is very suitable for vine cultivation, both for white and red varieties.



The bioclimatic index (I.B.V.) refers to the interaction of temperature, light and humidity (Constantinescu et al.,1964); the values recorded for the years under study are specific for this area (10-15), even higher, being beneficial for this culture.

The oenoclimatic aptitude index (IAOe) formulated by St. Teodorescu (1978) is being used for establishing the degree of climatic favorability that a region dispose for grape anthocyanins synthesis - for the production of red wines (Pop, 2003). The values of IAOe 2011-2012, as illustrated in table 1, confirm that the area is suitable for obtaining semi-dry and semisweet red wines (values beyond 4600 are indicated), with controlled denomination of origin.

The de Martonne aridity index allows estimating the degree of aridity of an area for a limited period (one year or one month). The average value calculated for 20 years classifies the viticultural center on the border between semi-humid and semi-arid climate, the years under study (2011 and 2012) being characterized by droughts alternating with rainy periods (table 1).

Table 1. Synthetic ecological indexes values compared to the multiannual average

Synthetic ecological indexes	Values		
	Multiannual average 1991-2010	2011	2012
IHr	3.02	3.2	4.1
I.B.V.	13.2	15	15.6
IAOe	5093	5351	5840
Aridity Index (de Martonne)	23	13	22

#### Soil hydric regime

In terms of soil moisture, in 2011 the initial water reserve value is situated well above the average, favoring the entry into vegetation, but since June, when the plant needs for water is still high, there is an aggravation of water deficit, with repercussions on the development of shoots and grapes (Fig. 1), having though a beneficial effect on sugar accumulation.

In 2012 the initial water reserve recorded a moderate deficit, approaching the normal in May and then registers a strong deficit, with negative effects on vines. The deficit recorded during the summer months is though normal for this time of the year for this area and favours sugars and polyphenols accumulation.

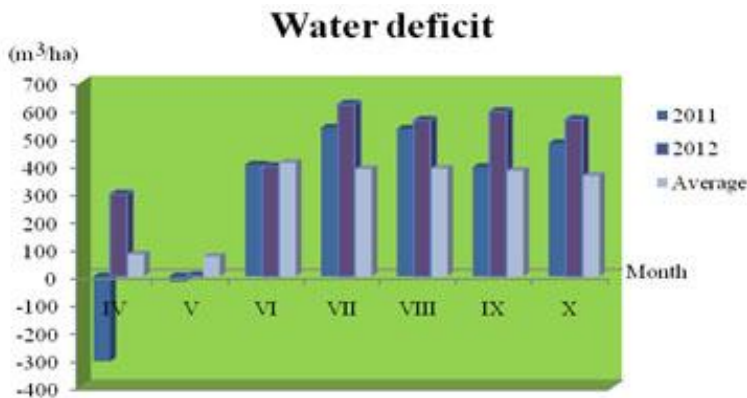
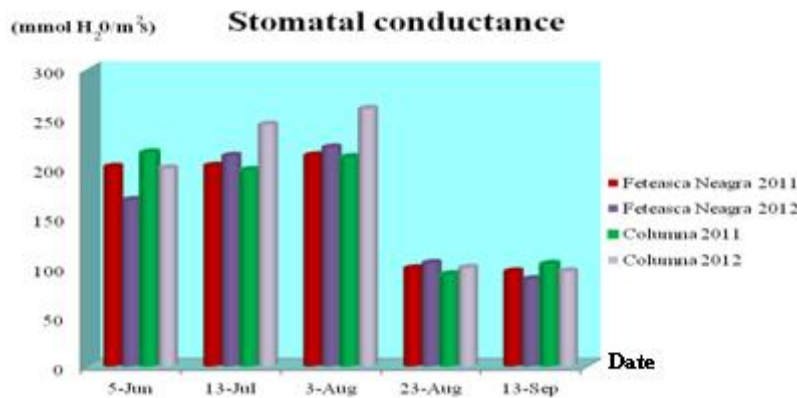


Fig. 1. Water deficit (m³/ha) during the vegetation period

Dynamics of the main physiological processes

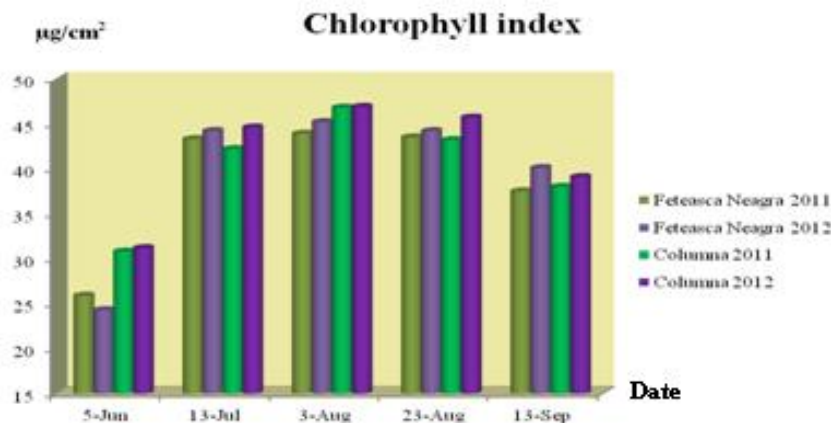
In 2011, the stomatal conductance for the two varieties under study recorded higher values at the beginning of the flowering phenophase, in comparison to the values obtained in 2012 for the same period. For Columna variety in 2012 the values were higher at the beginning of the veraison phenophase (259,8 mmolH<sub>2</sub>O/m<sup>2</sup>s), due to temperatures over 37 °C compared to the optimal transpiration intensity at 25-30 °C, indicating the intensification of transpiration process and stomatal closure. The values decreased significantly during grape maturation due to the slowing of vegetative growth processes.

The intensity of gas exchange measured by stomatal conductance indicates high values (212, 4 and respectively 243,8 mmolH<sub>2</sub>O/m<sup>2</sup>s) for both varieties starting with the veraison phenophase in 2012, due to the high temperatures in July and August (Fig.2).



**Fig. 2.** Stomatal conductance (mmolH<sub>2</sub>O/m<sup>2</sup>s) for Feteasc Neagr and Columna varieties during 2011-2012, starting with the flowering phenophase until full maturity

The values recorded for the chlorophyll index increase (43,3 and 44,2 µg/cm<sup>2</sup> for Feteasc Neagr and respectively 42,2 and 44,6 µg/cm<sup>2</sup> for Columna variety) starting with the berries growth period and sugars accumulation, reaching a maximum in early August (the chlorophyll content in leaves doubles - 45,7 µg/cm<sup>2</sup> on average) due to the intensification of photosynthetic activity, and then decrease during the ripening process (Fig. 3).



**Fig. 3.** The chlorophyll index (µg/cm<sup>2</sup>) for Feteasc Neagr and Columna during 2011-2012, starting with the flowering phenophase until full maturity

The values obtained for the concentration of chlorophyll pigments in 2012 (43,5 µg/cm<sup>2</sup> on average), were higher comparative to the previous year (37,5 µg/cm<sup>2</sup> on average), favoring the superior accumulation of sugars for both varieties under study, as will be observed in Fig. 5. During grapes ripening, regardless of the variety particularities, the concentration of chlorophyll diminishes, a process related to the depression of growth processes.

### Phytosanitary status of plantations

The evolution of the characters regarding the emergence and development of mildew, powdery mildew, gray mold and grape moth *Lobesia botrana* were monitored.

Vine mildew and gray mold manifested with greater intensity in 2012 compared to the previous year, due to climatic conditions favorable for the development of these pathogens (Heavy precipitation in May - 145 mm). The symptoms of these diseases manifested in both of the studied plots, the average infestation being between 2 and 7, from moderately resistant to resistant. Pest control was carried out by a complex of technological and ecological measures, with the mention that the treatments play a preventive role, and therefore have been applied during the incubation period of the pathogens (*Plasmopara viticola* and *Botrytis cinerea*).

The attack of the powdery mildew (*Uncinula necata*) recorded a higher intensity in 2011, the average infestation ranging between 7 and 13, from moderately resistant to moderately susceptible, and manifested on the young bunches in both varieties under study, Columna variety being affected in a higher proportion.

Manual works: correct tying of the shoots, thinning and partial defoliation created optimal conditions for good hub ventilation and effectiveness of the applied treatments.

Preventive combating of grape moth was achieved by applying green works and by placing pheromone traps (fig. no.4), which helped establishing the population density. The number of moths captured weekly ranged between 2-7 individuals/trap, the economic damage threshold of 100 butterflies/trap/week was not reached in neither of the two years under study. Under these conditions the application of a treatment is not necessary.



**Fig. 4.** AtraBot pheromone traps placed in the experimental plots

### Production quality

The quality of the grapes, appreciated by the sugar content, total acidity and grain weight, is presented as it was monitored over the ripening process, from the 6th of August to harvest time; from the obtained graphics (Fig. 5) the date on which the varieties reached full maturity, the grain weight is maximum and the sugar content optimal for starting the harvest can be easily observed.

The differences in terms of grape quality were not significant during the two years under study: the sugar content increased slightly - by 7-10 g/l, the grain weight - by 4-14g and the total acidity decreased by 0,4 - 0,6 g / l; it is worth mentioning the advance of full maturity and harvest time by 5-6 days in 2012, phenomenon driven by the global warming process and water shortage.

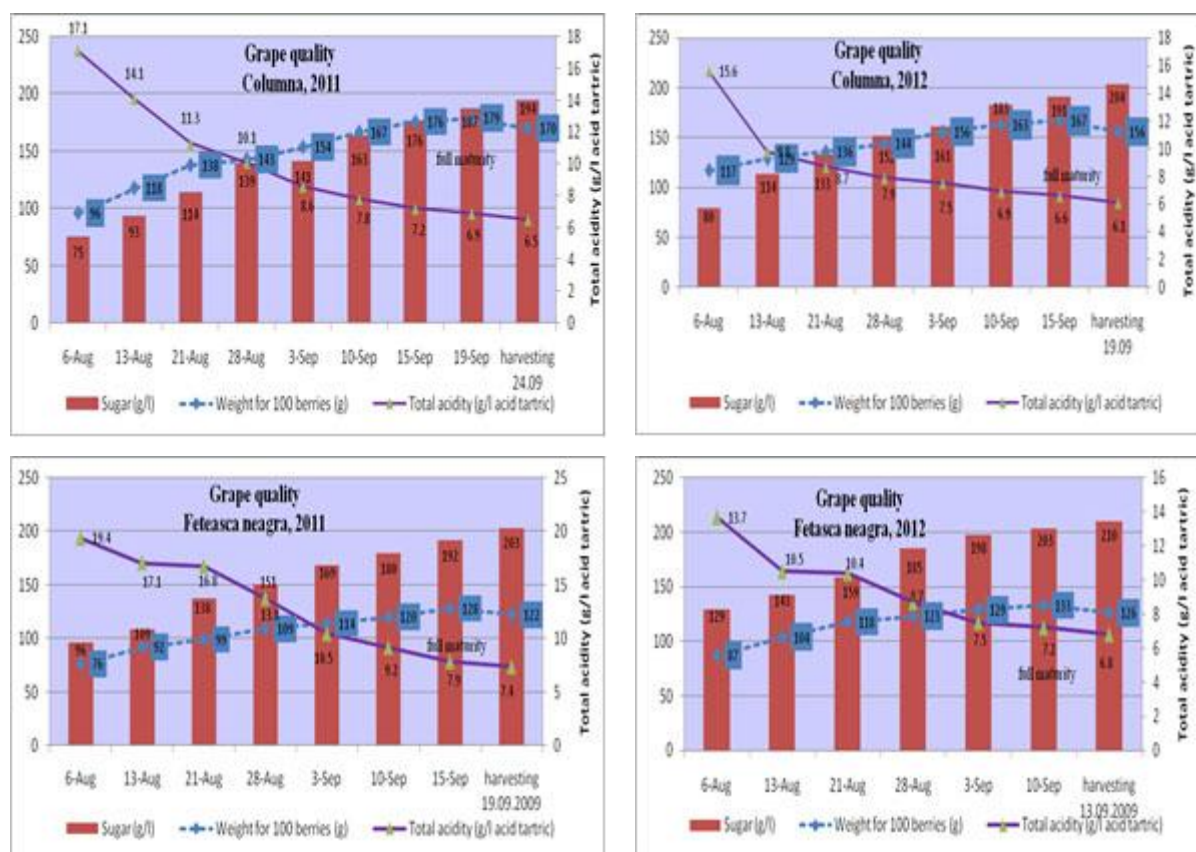


Fig. 5. Maturation dynamics of organically grown grapes

Physico-chemical composition of wines was evaluated based on the overall composition parameters (alcohol concentration, total acidity, volatile acidity, reducing sugar, non-reducing extract), by standardized physico-chemical methods. The analysis results are presented in Table 2. The resulting organic wines were extractive (19,5-26,8 g/l), with a normal alcoholic content (11,2 - 12,2% vol), balanced in terms of total acidity, qualitative wines classified in the controlled denomination of origin category (DOC).

Table 2. Physico-chemical parameters obtained for the organic wines obtained at the Murfatlar Viticultural Center

Year	Variety	Alcoholic content (% vol)	Reducing sugars (g/l)	Nonreducing sugars (g/l)	Total acidity (g/l tartaric acid)	Volatile acidity (g/l acetic acid)
2011	Columna	11,2	3,6	19,5	6,61	0,49
	Feteasc Neagr	11,8	2,5	26,7	6,18	0,55
2012	Columna	11,8	1,8	21,9	5,73	0,32
	Feteasc Neagr	12,2	3,2	26,8	5,35	0,56

### Conclusions

Murfatlar viticultural center climate, characterized by the climatic indexes values, is favorable for the ecological cultivation system of the varieties Feteasc Neagr and Columna.

The observations regarding the values of the stomatal conductance and chlorophyll content showed a good correlation between the intensity of transpiration and stomatal opening relative to the vine vegetation stage.

The cultivation technology has been adapted to the Murfatlar vineyard climate and was applied so that organic viticulture principles to be followed strictly, minimizing the intake of allowed plant protection substances, and the removal of those forbidden. Mechanical and manual work had the aim of optimizing treatment effects and minimizing the risk of pest and disease attack.

Sugar accumulation was slightly stimulated by climate warming and the titratable acidity at harvest time recorded low values, due to the frontloading of grapes maturation phenophase, the combustion of the organic acids being increased at higher temperature values. The obtained organic wines were classified in the category of controlled denomination of origin wines.

The climate conditions changing in the recent years and the tendency to maintain it as favourable for all grape varieties can be considered prospective for cultivation of the land in the ecological system.

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## THE INFLUENCE OF CONVENTIONAL AND BIOLOGICAL SYSTEMS CULTIVATION OF PLANTS ON EDAPHIC BIODIVERSITY

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### Abstract

One of the main goals of biological agriculture is to maintain the agro-eco-systemic balance, mitigation of negative impacts and improve environmental qualities. The realization of this objective is associated with agronomic aspects of soil fertility management, the dynamics of organic matter and the presence of edaphic fauna in the sub-soil layer. The keeping of a good level of soil fertility, which supports long-term productivity of agro-eco-system, depends on the choice of cultural techniques. Dynamics of organic matter in soil is influenced by many factors, among which the presence of micro-flora and micro-fauna of the land. The diversity of species of micro-organisms present in the soil performs important keys functions in the eco-systemic recycling organic matter, transformation of nutrients and environmental qualities of agro-eco-systems. The diversity of microorganisms presents in soil depending on the system of cultivation, the type of soil, plant species and other organisms present in agro-eco-system. Conventional agriculture, through cultural techniques used, poor soil biodiversity, in particular that rhizospheric, changes structural balance of microbial populations. The study analyzes the levels of terrestrial biodiversity in the two types of agro-eco-systems (biological and conventional), planted with the same crops, through the study of the connections between plants, soil and microorganisms, in order to define the differences between them and impact on fertility of soil and biological diversity.

**Key-words:** soil biodiversity, micro-fauna, edaphic fauna, agro-eco-system

### Introduction

The soil in agricultural systems represents the natural irreplaceable resource, which through its physic and chemical qualities and especially biological characteristics, accomplishes a range of fundamental functions, not just from the production point of view but moreover from the environmental point of view. Fundamental functions like biomass production, nutrients recycling, maintaining of fertility and filtering of different chemical pollutants could not be accomplished without an acceptable level of soil biodiversity (edaphic fauna). The choice of cropping system is fundamental to maintain this biodiversity and its functions. The density of present microorganisms in soil and their biomasses vary according to the soil and plants type and according to the cropping techniques applied. The diversity of microorganisms inside of an ecosystem is so a key element to maintain a healthy situation of agricultural soil (Borneman, 1996).

Setting of suitable equilibrium of soil biodiversity is reflected on environmental qualities of a cropping system. Conventional cropping systems based on monoculture and use of pesticide and herbicide, can impact the soil biodiversity and especially the biodiversity of rhizospheric ecosystem, changing the structural equilibriums of soil microorganisms communities (Bolton et al., 1985; Doran, 1980; Ramsay et al., 1986).

The composition and structure of soil communities is dependant not just from interaction among presented species and plants but from the chemical and physical nature of soil (soil structure, moisture, pH, temperature and nutrients), which impact the microbial life and select the most suitable microorganisms (Garbeva et al., 2004). Different studies have identified existing linkages

between soil biodiversity and its functions (Nannipieri et al., 2003). Though the environmental factors and soil typology impact the microbial diversity of soil (Girvan et al., 2003), frequently it is the typology of cropping practices used or the type of treatments applied that could determine obvious changes of biodiversity (Gomez et al., 2006), with consequences that are difficult if not impossible, to be recovered (Mocali et al., 2008).

### Materials and method

This study analyses two cropping systems (biological and conventional) planted with corn (*Zea mays*) in Shijak, near Durrresi, accomplished during 2011, on an area of 0.5 ha. In biological cropping area 90.2 ha) compost is used as nutrient. Another area (0.3 ha) close to the first one is managed with conventional practices. Fertilizers (ammonium nitrate) and pesticides are used in this area. The indicator chosen to evaluate the soil is the community of edaphic fauna. Biological analysis of soil through the study of micro fauna community serves as argument to verify differences between two types of agro ecosystems (Parisi, 2001). The level of diversity for samples taken is analyzed through the indicator QBS (Parisi, 2001).

In each plot samples are taken according to the standard methodology taking soil from both cropping systems. In these soil samples the presence of micro arthropods is analyzed through Berlese-Tüllgren selector. The samples are analyzed in laboratory to identify the respective classes according to the cropping system. The biological quality of soil is evaluated through QBS indicator (Parisi et al., 2005; Menta et al., 2008). The differences of biodiversity values (based on the calculation of Shannon-Wiener diversity indicator  $-H'$ ) are evaluated through the analysis of variance (Anderson, 2001; Anderson, 2005).

### Results and discussion

The analysis of micro arthropods populations, in both cropping systems, evidences the presence of different groups of them in the underground strata. In the plot managed biologically and where manuring is used, a higher presence of these groups compared to conventional cropping is identified. Higher values of QBS indicator are identified also, based on the evaluation of the diversity indicators Shannon-Wiener  $-H'$ .

Table 1. The evaluation of diversity indicators of edaphic fauna

Cropping system	Indicators			
	No. of biological groups	Density (individuals/m <sup>2</sup> )	QBS	Shannon-Wiener $-H'$ .
Biological cropping	14	33700	88	1.7
Conventional cropping	10	23200	43	1.1
Loss of diversity	4	10500	51 %	61.7 %

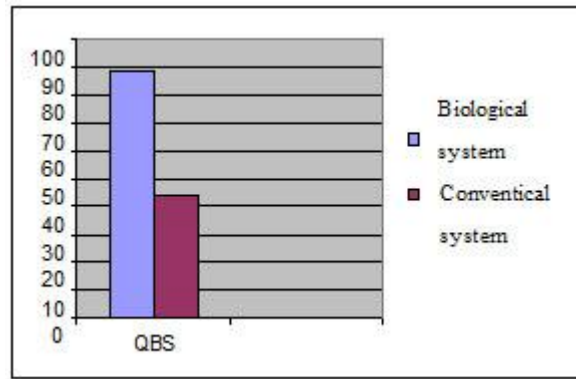


Figure 1. Values of QBS for both cropping systems in different treatments

From the above shown data it can be seen that there are high values of QBS indicator for corn crop in biological system of cultivation. A higher presence of systemic groups of arthropods in the biological system is due to the fact of manure application with quantities around 20-40 t/ha and leaving of after harvesting plants residues in field. They being transformed by micro organisms, increase the quantity of organic matter. In the plot where fertilizers were applied a lower number of micro organisms are seen. This situation is closely related to pesticide use and taking of almost all crop biomass as livestock feed also in this plot.

### Conclusions

The analysis of different biodiversity indicators that give clear evidence of higher presence of soil microorganisms in the plot of biological system sustained by standard manuring and other sustainable practices like green manure, leaving plant residues in field after harvesting, minimal tillage etc. tells us for the sustainability of this system and the decrease of negative impacts. The indicator of soil biological qualities (QBS) which is higher for the biological system confirms the positive effects of this system.

Low values of QBS in the conventional system (51% lower compared to biological system) show that this system through management practices used brings negative impacts regarding soil microorganisms and soil biological qualities.

The data offered by this study are useful because they evidence the fact that sustainable practices used by biological agriculture bring positive impacts in sustaining soil resources and inner functional equilibriums of the system.

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## **4. ENVIRONMENT PROTECTION AND NATURAL RESOURCES MANAGEMENT**

## CONSERVATION OF BIODIVERSITY IN ECOSYSTEMS OF THE BOREAL FORESTS

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### Abstract

Biodiversity in forest ecosystems is the main indicator of the stability and sustainability of the system. It is also a sign of ecological community orientation to a state of stable fluctuated equilibrium - homeostatic state. Implementation of any forest management activities must be accompanied by the trend of natural systems tending to a stable state. In Russia forest resources are mostly relates to a commercial forest (intended for logging). Important element of biodiversity conservation program in forest ecosystems is the preservation of the natural gene pool during all management activities. Long-term studies on stationary objects (since 1929) in the North-West Russia indicate that during harvesting with preservation of young retained undergrowth at the same time we preserve not only the undergrowth but the understory and ground cover also. Quantitative changes of biodiversity in forest ecosystems, as a consequence of any management activities, are just some of the intermediate indicator of changes in forest biogeocenosis, but do not explain their dynamics. Only perennial detailed observations of the dynamics of vegetation changes, at different stages of succession, provide a complete picture of nature and process of returning the system to homeostatic state.

**Keywords:** biodiversity, homeostasis, forest ecosystems, forest management activities, experimental objects with long-term observations

### Introduction

The problem of biodiversity conservation is particularly acute in occasions of intense human impact on forest ecosystems. A large amount of forests, in the forest fund of Russia, belongs to a commercial forest. It means that there is allowed to carry out all types of logging operations, reforestation and water conservation. Important element of biodiversity conservation in forest ecosystems is to keep the original, natural gene pool, in the performance of any management activities. First of all it concerns the undergrowth - the young generation of main forest species.

### Objects and methods

The experimental work was conducted since 1929, during 85 years, and it considered variety of experiments located in North-West of Russia.

The objects of research were mixed spruce-birch stands. In the various stages of their growth, different intensity of thinning cuttings was carried through. The studies covered four test sections and one control section, in witch there was no any forest management activities (Table 1).

Table 1. General characteristics of the objects of study “PP1”, 2012

General characteristics	Control section (A)	Test sections			
		B	C	D	E
The composition of the stand	<b>I</b> 10 + + <b>II</b> 10	9 1	9 1	8 2	10 +
Relative completeness	0,9	0,5	0,5	0,5	0,6
Timber volume, m <sup>3</sup>	<b>I</b> 348 <b>II</b> 148	301	331	321	432
Condition types	2	2	C2	C2	C2
The intensity of thinning cuts,%	-	15-24	25-34	35-45	over 45

( - *Silver birch*; - *Trembling aspen*; - *Norway spruce*; C – *Scots pine*; **I** – *first layer*, **II** – *second layer*)

Differentiation by the age, height, and diameter is typical for the mixed stands. And that is the reason of their horizontal and vertical differentiation [5].

Accounting of undergrowth, underbrush and ground cover was performed by selectively statistical method: the circular areas of 10 m<sup>2</sup> were placed one by one, touching each other, in three or more different rows, throughout the experimental objects. The sample magnitude must be at least 2% of the total subject area (Grjazkin, 1997), but because the studies were performed on stationary objects, some sections were enumerated completely. During that accounting procedure, special attention was paid to their qualitative and quantitative characteristics which directly, or indirectly, was the indicator of dynamic processes and the state of forest ecosystems.

### Results and Discussion

Our results showed that not only the structure of forest communities were under the influence of thinning cuts, but also were the microclimate and the environmental conditions [1, 2, 3, 4, 5, 7, 8]. The main out come of the thinning cuts was releasing of a part of the soil resources, which resulted in the extra growth of ground cover, soil protection and resource-saving. Those structural changes of forest communities aimed to restore the homeostatic state of the system. This implies that those extra soil resources are fixed in the biological cycle throughout enhanced growth and development of the lower vegetation layers (ground cover).

Thinning cuts were conducted in different intensity from 1929 to 1973, at 4 of those 5 test sections. Accounting of the understory vegetation barred 5 years after the last cycle of thinning cuts, showed that the implementation of a strong (35-45%) and very severe thinning cuts (over 45%) leads to an increase in the proportion of shrubs and subshrubs, compared to the control section. Also, at these facilities, there was noticed an increase in the proportion of ground cover, cranberries and raspberries, which in turn improves the habitat conditions (Belyaeva, 2011). Along with the development of the moss layer after strong and very strong thinning cut, active growth of heliophyte grass cover is also observed. This is the expected response of forest ecosystems to a strong anthropogenic influence, and it also indicates the increasing intensity of the biological cycle.

The results of observations made in the course of 15-20 years after the last cycle of thinning cuts show that the proportion of subshrubs, grasses and mosses, corresponds to parameters in the control section, and that prove that ecosystem has recovered and returned to a state of a stable fluctuated equilibrium.

Comparative quantitative analysis of biodiversity undergrowth, bushes and ground cover in the control section, and the sections in which thinning cuts of varying intensity was conducted, shows

that the medium and low intensity of thinning cut lead to a decrease in the number of species. In the other hand, their high intensity (of about 50%), generated their increase (Table 2).

Table 2. Biodiversity of understory vegetation on the experimental object “PP1”, 2012

Section	Intensity of Thinning cuts,%	Biodiversity, number		
		Undergrowth	Underbrush	Ground cover
	Control section	3	1	29
B	15-24	2	2	24
C	25-34	3	2	24
D	35-45	3	1	21
E	>45	4	2	30

This is primarily due to the appearance of ruderal species. At the same time, there is also a relatively high biodiversity of all structural elements of the understory vegetation on other sections. The reason for this is the fact that the last thinning cuts was carried out 40 years ago, and during that time all the sections have already come back to a state of homeostasis.

The structure and the biological characteristics of the control sections is the same as the original state of all the other sections was before starting thinning cuts in 1929. Consequently, the birch prevails in the main layer and spruce is present only in the second layer. After thinning cuts in sections B, C, D, E spruce become edicator, with the 80% participation in the first layer. This means that thinning cuts caused changes of the environmental conditions. It follows that the quantitative analysis of biodiversity cannot give a complete picture of the dynamic processes at these facilities.

Environmental changes that have occurred as a result of thinning led to qualitative and quantitative changes in ground cover. The study of ground cover was focused on identifying its species diversity, and the projective cover. After thinning cuts, the share of helophytes and shrubs, comparing to the control section, increased. But after 20 years that proportion came close to the original state of dynamic equilibrium (Belyaeva, 2011, 2006; Melnikov, 2006). The results of studies in 2012 showed that the total projective ground cover is the largest in section E \*, and the smallest in section A (Table 3).

Table 3. Characteristics of the stand and ground cover on different sections of experimental object “PP1”, 2012

Section	Composition of stand	Completeness	Average age, age	Total projective cover of ground cover, %	Share of ruderal species of ground cover, %
	10 +O +	0,9	123	110,8	18,9
B	9 1	0,5	123	142	5,6
C	9 1	0,5	123	137,8	8,9
D	8 2	0,6	123	140,6	3,8
	10 +	0,7	123	161,4	4,3
*	10 +	0,7	123	173,1	9,1

( - Silver birch; - Trembling aspen; - Norway spruce; C – Scots pine)

The main reason for expansion of ground vegetation is the fact that the section E \* was completely cleaned of underbrush in 1994, and also the thinning cut of high intensity (over 45%) was

performed. The reason for the relatively small projective ground cover in the control section, most likely, is a well-developed second layer of spruce, as well as the relatively high number of undergrowth of spruce that shade near-surface layers. An unexpected result was obtained in section A. The reason for those results was that the second layer of the undergrowth of spruce was clump (group) located. Therefore, the certain number of glades occurs in the canopy of birch, and they were the niches for ruderal species, which possessed 18,9% of all ground cover (Table 3.).

Dynamics of undergrowth and ground cover determined changes in the stand, and were responsible for the reforestation processes in the forest ecosystem. The success of the thinning cuts of varying intensity can be estimated by a large number of signs, such as changes in the stock and structure of forest stands, forest conditions, and the composition and morphological (qualitative and quantitative) characteristics of the forthcoming reforestation.

An important factor in that reforestation after thinning cuts is a competitive correlation between underbrush and undergrowth. Changes in environmental conditions after thinning cuts at some stage causes the appearance of the fast-growing underbrush, particularly rowan. According to the account in 2012, with an increase in the intensity of thinning cuts, rapidly increased the number of rowan, with the exception of section E, where the intensive thinning cuts of underbrush in 1994 was conducted as a measure to encourage natural regeneration (Table 4).

Table 4. Characteristics of understory vegetation on experimental object “PP1”, 2012

Section	Intensity of thinning cut, %	Strength, number/hectare		Share of a large, %		Total projective cover of ground cover, %
		Undergrowth of Spruce	Underbrush	Undergrowth of Norway spruce	Underbrush	
	Control	3748	9729	31	37	110.8
B	15-24	6499	6884	3	72	142.0
C	25-34	3292	5666	9	64	137.8
D	35-45	9549	10350	1	97	140.6
E	>45	4520	4680	26	56	163.4

This study showed that the distribution by size categories - the underbrush on the one, and regrowth on the other hand, is inversely proportional. That is, the larger the underbrush is, the smaller regrowth is.

Researching results also indicate that the 35-45% intensive thinning cut provides the best spruce regeneration. It should be emphasized that it also encourages the widening of the underbrush to a large degree, resulting that large undergrowth is practically missing. On the other side on the section E, where the intensive thinning cutting of underbrush was conducted, a large amount of high quality spruce undergrowth appeared (Table 4).

### Conclusion

Quantitative changes of biodiversity in forest ecosystems, as a consequence of any management activities, are just some of the intermediate indicator of changes in forest biogeocenosis, but do not explain their dynamics. Only perennial detailed observations of the dynamics of vegetation changes, at different stages of succession, provide a complete picture of the nature and process of returning the system to homeostatic state.

Conducting the thinning cuts of the medium intensity (30-40%), together with the subsequent regulation of the amount of underbrush, provides the growth of the high quality stands in the future. A predominance of commercially valuable species allows their subsequent successful natural

regeneration. The regular implementation of these activities does not contradict the ecological processes in the forest ecosystems, and the system stability (homeostatic state) will be rapidly achieved.

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**LEGISLATION IN SERBIA IN THE FIELD OF COLLECTING  
MEDICINAL AND AROMATIC PLANTS**

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**Abstract**

According to the international criteria of the International Union for Conservation of Nature (IUCN) and the World Conservation Monitoring Centre (WCMC), Serbia together with the mountain areas in Bulgaria is one of six European and 153 world centers of biodiversity. In this region it has been recorded the presence of a high percentage of rare, sparse or endangered plant and animal species, of which 1,600 have the status of internationally important ones for the global preservation of genetic fund and biodiversity. Protection of the medicinal and aromatic plants' resources in our country is under strict legal control, which preceded by the ratification of appropriate international conventions (Agenda 21 - Rio Declaration, United Nations 1992, Bern Conventions 1982, Council Regulation No. 338/97 - 1996). The Government of the Republic of Serbia by the legal and regulatory regulations has prescribed different levels of protection for medicinal herbs and authorized the Institute for Nature Protection of Serbia to establish by the open competition the contingents of medicinal herbs that can be collected in the current year. Complete legislation has been passed by the Government of the Republic of Serbia, and they take effect upon their publication in the Official Gazette of the Republic of Serbia. The collection of wild flora and fauna is allowed upon getting a permit for collection of protected species in the permitted quantities as well as in the prescribed periods. The SEED study (2003) on the situation of herbal sector in Serbia and Montenegro has shown that despite the great wealth of natural resources in medicinal and aromatic plants (MAP) (hence the significant export potentials), a number of species and many populations, depending on the region, require special attention as they face pressures of excessive and/or improper exploitation.

**Key words:** medicinal and aromatic plants, protection of resources, legal documents, permits

**Introduction**

The problem of over-exploitation of medicinal plants *in situ* is a threat to the natural resources, so it is necessary to preserve the biodiversity of medicinal plants in Serbia. This requires proper assessment of risks to biodiversity, and all that is necessary in order to reconcile both the acquisition of income and preservation of natural resources (Parotta 2002). In order to develop this sector it is necessary to have cooperation in preserving MAP species by pointing to the collectors onto benefit of rational exploitation and cultivation. In this activity it should be point to the consequences of the biodiversity loss for the collectors and producers as well as on an importance of biodiversity for the community. (Gadgil, Berkes, Folke 1993)

According to the international criteria of the International Union for Conservation of Nature (IUCN) and the World Conservation Monitoring Centre (WCMC), Serbia together with the mountain areas in Bulgaria is one of six European and 153 world centers of biodiversity. In this region it has been recorded the presence of a high percentage of rare, sparse or endangered plant and animal species, of which 1,600 have the status of internationally important ones for the global



preservation of genetic fund and biodiversity. Given the availability and accessibility for commercial exploitation, the medicinal herbs have been classified into two groups: plants that are widespread in nature and types of medicinal and aromatic plants which are under state protection.<sup>11</sup>

<sup>12</sup> Among many species of wild plants in Serbia in addition to endemic ones, there are species that have not been sufficiently investigated. Although the territory of Serbia is characterized by high floristic diversity (over 3,500 plant species and subspecies), as well as by the wealth of natural MAP resources (more than 700 species are used in ethno-medicine), due to irrational use some types of medicinal and aromatic plants have become extremely endangered and rare: *Gentiana lutea* - yellow gentian, *Gentiana punctata* - gentian, *Adonis vernalis* - wild flowers, *Arctostaphylos uva ursi* – bearbeery, *Menyanthes trifoliata* - bitter clover, *Cetraria islandica* - Iceland moss, *Sideritis scardica* - sideritis, *Gypsophila paniculata*, some species of genus savory, orchid, etc. Sustainable development of natural resources of medicinal and aromatic plants is directly dependent even on the application and improvement of regulations and standards that have to be harmonized with EU laws and standards.

The SEED study (2003) on the situation of herbal sector in Serbia and Montenegro<sup>13</sup> has shown that despite the great wealth of natural MAP resources (the significant export potentials), a number of species and many populations, depending on the region, require special attention as they face pressures of excessive and/or improper exploitation.

A coordinated approach to the conservation of medicinal and aromatic plants' biodiversity is carried out through a number of programs aiming at the germplasm conservation at national and international level, according to the recommendations of international organizations, such as WWF, IUCN, WHO, IPGRI and others (Heywood 1989). Since 1993 there has been started in our country with introduction and application of legislative regulations in this area. Appropriate measures have been proposed by a number of institutions, in particular by the Institute for Nature Conservation of Serbia, Ministry of Agriculture and Ministry of Science and Environmental Protection. It is in the procedure the adoption of the appropriate law as well as the series of by-laws as a continuation of the EU harmonization process.

### Legislation and Standards

Protection of the medicinal and aromatic plants' resources in our country is under strict legal control, which preceded by the ratification of appropriate international conventions (Agenda 21-Rio Declaration, United Nations 1992, Bern Conventions 1982, Council Regulation No. 338/97 - 1996). The collection of wild flora and fauna is allowed upon getting a permit for collection of protected species, in the permitted quantities as well as in the prescribed periods.

The Government of the Republic of Serbia has prescribed different levels of protection for medicinal herbs and authorized the Institute for Nature Protection of Serbia to establish the contingents of medicinal herbs that can be collected in the current year by the open competition procedure. Legislative acts that have to be applied by producers and processors of medicinal plants are the following: laws, regulations, rule books and orders. Complete legislation has been passed by the Government of the Republic of Serbia, and they take effect upon their publication in the Official Gazette of the Republic of Serbia.

The field of medicinal and aromatic plants is regulated by the following **laws**: The Law on Plant Protection ("Official Gazette of SRY", No. 24/98, 26/98, 101/05 and 41/09); The Law on Environmental protection ("Official Gazette of RS", No. 135/2004 i 36/09); The Law on Organic

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<sup>11</sup> Study "Market Analysis for the Products of Higher Degree of the Medicinal Plants Processing (The First Phase), Market Analysis of Medicinal and Aromatic Plants and the Plant Based Products (Basic Trends) – Institute for Market Research

<sup>12</sup> "Official Gazette" RS, No. 50/903 i 16/96

<sup>13</sup> www.balkanherbs.org

Production and Organic Products "Official Gazette of SRJ", No. 62/06); The Law on Plant Health "Official Gazette of RS", No. 41/09); The Law on Plant Chemicals ("Official Gazette of RS", No. 41/09); The Law on the Protection of the Plant Breeders' Rights ("Official Gazette of RS", No. 41/09); The Law on Nature Protection ("Official Gazette of RS", No. 36/2009 i 88/10); The Law on Food Safety in Republic of Serbia ("Official Gazette of RS", No. br. 41/09).

The particular **Regulation** specifies the species of wild flora, fauna and mushrooms, i.e. the protected species whose collection from natural habitats, utilization and sales have been brought under control and determined the amount of compensation for their use: the Regulation on controlling the use and trade of wild flora and fauna ("Official Gazette of RS", No. 31/05, 45/05, 22/07, 38/08, 9/10).

Among the **Rule Books** governing the issue of LAB, there can be mentioned the following: The Rule Book on the methods of organic plant production and organic production methods of wild plant and animal species from natural habitats ("Official Gazette of RS", No. 47/09); The Rule Book on the cross-border sales and trade of protected species ("Official Gazette of RS", No. 99/09); The Rule Book on designation and protection of the strictly protected and protected wild species of plants, animals and mushrooms ("Official Gazette of RS", No. 5/10); The Rule Book on criteria for allocation of habitat types, on the habitat types, on the sensitive, threatened, rare and priority habitat types for protection, as well as on the protective measures for their preservation ("Official Gazette of RS", No. 35/10); The Rule Book on the compensation price list applied to determine the indemnity caused by the unauthorized act done in relation to the strictly protected and protected species ("Official Gazette of RS", No. 37/10); The Rule Book on conditions for establishing a gene bank for the wild plants, animals and mushrooms, on mode of the gene bank operating, on the handling with biological material, contents of requirements and documents required to be submitted with the application for a license for establishment of the gene bank ("Official Gazette of RS", No. 65/10); The rule Book on the conditions and methods for assessing the risk to carry out the phytosanitary inspection of the plants' consignments, plant products and regulated objects with lower frequency ("Official Gazette of RS", No. 67/11); Regulation on the quality of tea, herbal tea and their products ("Official Gazette of RS", No. 4/12).

The **Order** is made at the beginning of each year in late March or early April, e.g. The Order prohibiting the collection of certain protected species of wild flora and fauna in 2011 ("Official Gazette of RS", No. 24/11). This Order prohibits the collection of protected species of wild flora throughout the territory of the Republic of Serbia, e.g. in 2011 there were the following species: *Hypericum barbatum* Jacq. – the cilia Klamath weed, *Hypericum maculatum* Crantz – the mountain Klamath weed, *Hypericum rumelicum* Boiss. – the rumelian Klamath weed, *Iris pseudoacorus* L. – the marsh iris, yellow iris, wild iris, *Veratrum nigrum* L. – the black hellebore. The recommendation for all collectors is that they have to be familiar with the Order of the current year, in order to avoid coming into conflict with the law as well as the payment of penalty.

In addition to the legal acts regulating this issue, we highlight and **strategic documents** dealing with the subject of medicinal herbs: The Strategy of the Medicinal Herbs Protection in Serbia, The Institute for Research of Medicinal Herbs "Dr. Josif Pan ic" (1999); The National Strategy of Sustainable Development of Serbia (2008); The Strategy of Biodiversity in the Republic of Serbia for the 2011-2018 period ("Official Gazette of RS" No. 13/11); The Strategy of Agricultural Development of Serbia ("Official Gazette of RS" No. 78/05).

### **Legislation Procedure in the field of MAP natural resources protection in Serbia**

The license for collection of the wild flora and fauna protected species for the commercial purposes has been issued by the Ministry in charge of environmental protection on the basis of the public announcement. The public announcement contains types and amounts that can be collected in the current year. The license can be obtained by the legal entities and entrepreneurs registered for

carrying out the collection of forest and other products, their purchase, sales and processing. The condition for getting a license is that its potential carrier has not been convicted for misdemeanor of the regulations on the protected species' collection of wild flora and fauna. Together with requirement for issuing of the license, there have to be submitted the following prescribed documentation (data on the applicant, protected species, collected quantity, location of collection, collection centers, capacities – facilities, storage, processing, sales, as well as the proof of fee payment). The fee is paid at the annual level equal to 10% of the price for the particular specie, as determined by the competent Ministry before the public announcement for the license issuing. Entities that are licensed to collect protected species have obligation to submit in due time and on prescribed forms to the competent Ministry and the Institute for Nature Conservation of Serbia the data of the collection (by species and collection centers), as well as on utilization and sales. Those entities that are engaged in cultivation of the protected species are also required to submit to the Ministry the required data.<sup>14</sup> The collection, use and trade of protected species is placed under control in order to ensure their sustainable use, by preventing the collection of species from natural habitats in the quantities and in a manner that would endanger their survival in the future, which would violate the structure and stability of habitats. The total quantities of protected species collected for commercial purposes have been determined each year on the basis of previously assessed status of species on natural habitats. Also, this regulation prohibits the collection of plants from small population, pulling underground organs (roots, rhizome, etc..) for the species where the above-ground organs have been used, as well as the crushing and cutting of trees and branches of trees and shrubs if there have been collected the fruits, flowers or leaves, and collecting near busy roads and waste dumps.

Under the protection of the state it has been recently 8.09% of the flora of Serbia, and now it is 12.52%.<sup>15</sup> Implementation of legal regulations is the responsibility of the Institute for Nature Conservation of Serbia, which implements its protection program through annual quotas for each species. It is difficult to estimate the total amount of MAP which is harvested in Serbia. All together quotas amount to about 6,000-8,000 tons of fresh plant material depending on the particular year. From this quantity it could be got around 1,500-2,000 tons of dried herbs. It is not possible to get information whether all the quotas are exhausted or some of them are exceeded. The quotas that were relatively low in the 2000-2004 period, have been increased in the 2005-2007 period and in 2009. Annual quota has been increased from 201 to 2,000 tons for juniper, from 297 to 5,000 tons for wild rose, from 21 to 150 tons for wild strawberry, from 45 to 2,000 tons for wild blackberry, from 681 to 2,000 tons for blueberry etc.

Quotas would have to be reviewed every year, depending on the status of natural populations, in order that the number of individual specie unit would not have been in decline. As monitoring requires significant resources and engagement of competent teams of professionals, it is more practical to predict the vulnerability of species, taking into account the actual collection methods, areas from which the plants are collected and indicators, such as drugs (organs - parts that are collected), the profile of collectors and rough estimates of quantities, prices and trends.<sup>16</sup>

### Conclusion

In order to develop the MAP sector together with biodiversity preservation, it is necessary to have adequate application of legislative as well as cooperation of all stakeholders, particularly by emphasizing to the MAP collectors the benefit of rational exploitation and growing of MAP. In this process it is necessary to point at the consequences for collectors and producers coming from the

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<sup>14</sup> [www.zzps.rs](http://www.zzps.rs)

<sup>15</sup> USAID Agribusiness project in Serbia (2008): Herbs, mushrooms and forest fruit (medicinal and aromatic plants) value chain assessment, 34, [www.agrobiznis.net/.../Herbs\\_Mushrooms\\_and\\_Forest\\_Fruits\\_Value\\_Chain\\_](http://www.agrobiznis.net/.../Herbs_Mushrooms_and_Forest_Fruits_Value_Chain_).

<sup>16</sup> Cunningham A. B. (2001): Applied ethnobotany: people, wild plant use and conservation. Earthscan, London

loss of biodiversity, as well as at significance of biodiversity for the community. That is why the stimulating of organized plantation production through the subsidies given to the producers would encourage the collectors to begin with production of some imperiled species (gentian, calamus), as well as the species growing wild that have been introduced in cultivation (marsh mallow, plantain, yarrow). By better cooperation within existing association in the MAP sector it has to be assured the position of MAP collectors, as one of the key but marginalized social group in the chain of the MAP trade and processing.

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- [www.balkanherbs.org](http://www.balkanherbs.org)
- [www.zzps.rs](http://www.zzps.rs)

## THE ANALYSIS OF ENVIRONMENTAL COSTS IN THE FOODS RETAIL

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### Abstract

This paper, considering the meaning of the problem treated, deals with research of determinants of dynamics and specificities of the environmental cost structure-ecological costs, as well as their influence on performances in the food retail. Three significant categories of the environmental costs in the food retail are as follows: energy consumption, carbon dioxide emission, and water consumption. For purpose of optimization of influence of all, above stated, the most possible control is required.

**Key words:** Renewable energy, greenhouse effect gas emission, water, supply chain.

**JEL Classification :** D40 L81 M41

### Introduction

In this paper, considering the meaning of the problem researched, a special note is given to: meaning of the food sector, specificities of environmental influence to the food supply chain, components of the food costs, structure of environment costs of companies in the entire sector of food and beverage retail ( 500 companies within S&P ), meaning of the effective water consumption in the food retail, as well as in waste food treatment. Significant attention, in the context of the afore said is given to the strategies and techniques of the costs control in retail, as an important part of the food supply chain.

### Material and methods

*Literature* treating the research of effect on development and applying the concept of a sustainable development in the food retail is very abundant (Lukic,2012; Mattison 2013). One part of this literature also describes research on the specificities of the environmental costs in the food retail. All this literature available and used in this paper functions as the startpoint for theoretical, methodical and empiric base for performing an in-depth research of such complex problem: Influence of the environmental costs-ecological costs on their retail food performances.

The Initial *hypothesis* ( H1 ) of this research is : that environmental costs are becoming more and more significant determinant of the entire performances in the food retail. The second *hypothesis* ( H2 ) is in relation with the same is: Implementation of the appropriate strategies and control methods, can in great measure „optimize“ the influence of the environmental costs the performances in the food retail.

Methodology of the hypotheses given is based on the analysis theory, normatives, and especially on the comparative ( and ) descriptive analysis of the empiric data within the context of the issue researched in this paper.

Empiric data used for this purpose are mainly collected from the literature and web sites.

## Results and discussion

In a recent days, the global *growth of expenditures for food and drinks*, has been recorded ( USDA: Economic Research Service). Value are varying from country to country. Expenditures for food per capita in 2011 year ( calculated in US Dollars ) are as follows: USA – 2,329, United Kingdom-2,225, Germany- 2.658, France. 2,263, Slovenia-2,054, Italy-3,276, Japan-3,998, Hungary-1,317, Bulgaria-947, Rumania-1,564, Bosnia and Herzegovina-1,262, Macedonia-1,253 ( USDA –Economic Research Service). Consequently, the costs are significantly greater in the developed countries than in the non develeopd countries and countries in trasnsition.

Ecological certificates issuing ,becomes mainstream in the food sector.From the global view ,the *growth of organic food market is becoming a mainstream*. Such food is sold in the conventional shops (available in the supermarket chains), not only in the specialized and other shops. ( Brcic-Stipcevic, 2011;Papista, 2012; FiBL-AMI survey 2013, based on national data sources). According to the Symphony IRI Group research, the average prices of goods with a private brandname are 30% lower ,comparing with a natzional brandname.Retail prices are greater at the regular then the „healthy“ products.

At the global level, there is one third loss from the entire food produced for human diet, that is 1,3 billion tons per year ( Gustavsson, 2011).*Losses of food* are high both in the industrialized countries and the developing countries, where in the developing countries, more than 40% occurs in the retail sale and consumption( Gustavsson ,2011).

For purpose of optimization, the knowledge of the *costs structure of the food* through the entire supply chain.The figure 1 shows such structure on the example of the USA.

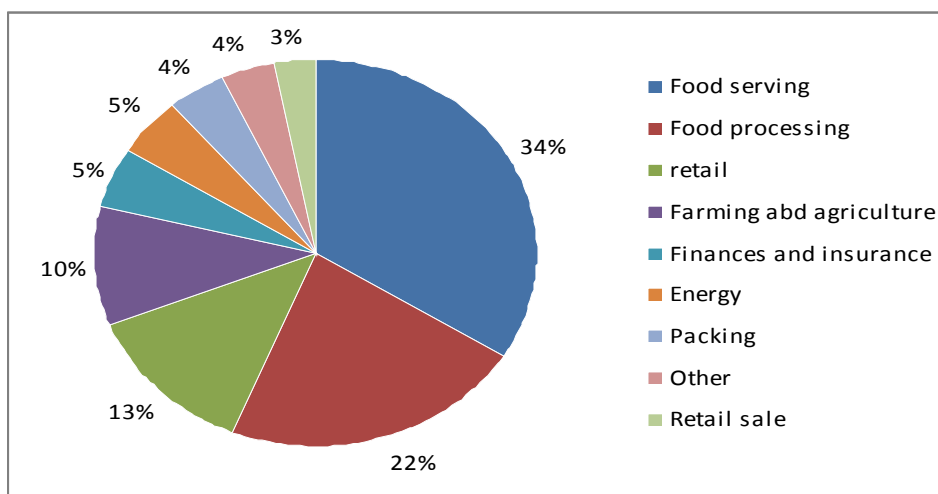


Fig.1 Components of the food costs in the USA in 2011 year

Note: Created by the author

Source : USDA-Economic Research Service

According to the data shown in the Figure 1, costs of retail sale in the USA in 2010 year, participated with 12.8 cents ( 13% ) per one Dollar of the sales price (100%). Participation of the trade costs ( wholesale and retail sale) in the retail price differ by the individual categories of the food. For example ( in April -13 2013) was 49,2 % for beef and 71,3% of pork (USDA-Economic Research Service).

Significant component of the food costs is the energy.*Energy efficiency* in the food sector is recognized through the supply chain.According to the Earth Policy Institute, consumption of the energy through the food value chain at the following stages is:Agricultural process-21%, food processing-16%, packing-7%, retail sale-4%.Currently, 9% of the total american energy

consumption is used for production, processing and transport of the food.(Foo Miles-the center for Environmental Education, www.ceeonlineorg>...>Upload Knowledge).

The leading author of the recently published food study Chris Weber from the Carnegie Mellon University, published by the Environmental Science & Technology, emphasizes that 83% of the greenhouse effect *gas emission* within the chain food value comes from the growing and harvest.Almost all phases of the food value chain are taking significant role in th carbon print.Emission of the greenhouse gas effect ( GHG ) ,by each phases in the food supply chain is as follows:Agriculture-40%, Fertilizer production-5%, Food producton- 12%, Packing-7%, Transport.12%, Retail sale-7%, catering-6%, in-house consumption-9%, and treatment( removal) and waste-2% ( Garnt, 2011).

The food sector in the European Community takes the first place in regard of incooms,with over 8 million empoloyees.*Logistics and chain supply control*, have a significant role in the food sector.Characteristics of the food supply chain differ for countries with the low, medium or highh Gross product ( Boye, 2013).

Integrated access to the designing and managing of the food supply chain includes simultaneous quality control, protection, sustainability and logistic efficiency from production and processing of the food, up to the entire food supplying chain,based on the principle „from the farm to the fork“ ( Manzini, 2013).

Inclusion of the sustainability in the food supply chain represents on of the significant changes having function of sustainable development realization, as well as growth of all members, including retail sale in 2011 year, the Agency of Enviroment in Europe published facts quoting that food and beverage sector participates in the global resources consumption with 23%, 18 % in the carbon dioxide emission,along with greenhoue effect, sour gases 31% ( Manzini, 2013).

Usage of the carbon print shows problems of influence of the food transport in regard of environment.Table 1 shows carbon prints through the life cycle in the milk production.

Table 1. Carbon prints in the milk production through the life cycle phases

Life cycle phases	Raw material production	Production/processing	Logistics/distribution	Retail sale	Usage in production	Recycling and removal
Carbon print	73%	9%	3%	10%	3%	2%

Source: Konieczny, ( 2013 )

Facts in the table above show that carbon prints in the milk retail sale are smaller than those found in production and processing.Carbon prints, however are differ from one food category to other.( Konieczny , 2013).

Food transpot becomes one of more significant sources of the grenhouse gas effect emission.It is quite obvious, due to a fact that 817 million tons of food are transported all over the world.“Food miles“, one of the leading ways for sustainability evaluation, i.e. recognition the environment effect ( Paseel, 2013).This term denotes food transport up to the final consumption point.The transport distance growth every year, nowdays it is 1,300 miles.

As regards energy consumption and carbon dioxide emission which products the greenhouse effect, the *transport model* is a factor of influence ( Table 2).Energy consumption and carbon dioxide emission which products te greenhouse efefct is the greatest in the air transport, and in same time, thgis emission is the lowest in the international water-container transpoort.A choice of the transport model ,the relative consumption can be optimized.By decreasing the grenhouse gas emission ,positive effect on the climatic changes will be obtained.

Table 2. Influence of the transport model on energy consumption and carbon dioxide emission

	megaJoules per ton-km	Kg CO2 eq per ton-km
International water-container	0,2	0,14
Internal water	0,3	0,21
Railway	0,3	0,18
Truck	2,7	1,8
Air transport	10	6,8

Source: Konieczny ,(2013)

Environment costs in the food and beverage sector as well as in the food retail sector are of great importance. The proofs of such statement is shown through facts given for companies dealing with the retail sale of food and beverage ( as part of S&P 500 companies), shown in the Table 3.

Table 3. Components of the environmental costs of the companies within the sector and food and beverage retail sale

Components	Food and bevarage ( in billions)	Retail sale ( in billions)
Water	\$ 38,398	\$ 8,009
Soil and water polution	\$12,019	\$ 7,409
GHGs	\$ 10,323	\$ 3,110
Air pollution	\$ 7,589	\$ 1,724
Waste	\$ 353	\$ 943
Total	\$ 68,682	\$ 21,195

Source: Mattison, ( 2013)

The data in the Table above show that environmental costs of the companies in the food and beverage sector are 69 billion US Dollars and 22 billion US Dollars respectively ( as a part of 500 S&P companies. Key components of the environmental costs in the Food and beverage sector are ,as follows: water consumption (\$ 38 billion), soil and water polution (\$ 12 billion), greenhouse gas effect emission (\$ 10 billion), an air pollution (\$ 8 billion). In regard of the food retail sale , the costs include the following items: Water consumption ( \$58 billion), GHG emission ( \$ 7 billion). Components of the environment costs are however different for each food category. The water consumption is a significant component of all. Better and average water consumption as the environmental costs components is an opportune solution. In the same time, the uneconomical consumption can only bring a risk ( Mattison ,2013).

Benefits gained by reducing food waste through the supply chain are of great importance ,for the entire society from one side, and for each company and any individual ,from the other side. ( Erikson, 2012). The Table 4 shows the results of such research.

Table 4. Assessment of the effects of food waste reduction throughout the supply chain in Sweden 2011th and 2012th

Supply chain sector	Waste (tons )	Waste per capita (tons )	Margin benefit for the society in regard of waste reducing ( SEK/kg)	Margin benefit for an individual or ecompany in regard of waste reducing (SEK/kg )
Household	67,500	72	81	62
Restaurants and caterings	125,000	13	31	12
Retail sale	39,000	4	25	6
Industry	171,000	18	25	6

Izvor: Eriksson, (2012)



All data from the Table above show obvious benefits of reducing food waste through the entire chain of the food supply, for the society, as well as for an individual, or company. They are more valuable for the society than an individual, or a company (In Store, June 2013, www.instore.rs).

Due to a complex ecological significance, there is a plea for the system of no returnable package, instead of returnable, due to a huge amount of money waste. This is shown in the research carried by Heineken.

Food recycling brings significant benefits. Therefore, recycling rate on the food supply chain members is increased by each year. Empirical results obtained in Japan confirm this statement (Takata, 2012).

### Conclusion

This paper stresses viewing of factors, specificity of a structure and effect of the environmental costs to the performances in the retail sale, as a member of the food supply chain.

In such context, it is concluded that the environment costs-ecological costs, make more significant factor of the costs efficiency, as well as for performances in the food retail sale. Their structure within the retail sale is specific in relation to the entire food sector.

Three significant components of the environmental costs in the food retail sale are as follows: Consumption of energy, greenhouse gas emission effect and consumption of water. With an efficient control, „optimization“, of the environmental costs structure, as well as their influence on the performance advancement in the food retail sale, can be significantly controlled.

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## OBSERVED CHANGES IN GRAPEVINE PHENOLOGY IN THE REGION OF SREMSKI KARLOVCI, SERBIA

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### Abstract

An analysis of recent-past changes in grapevine phenology in the region of Sremski Karlovci was performed. The study was based on 26 years of observations (1986–2011) on beginning of budburst, beginning of flowering, beginning of veraison and harvest for 21 different vine cultivars. The study revealed a trend toward earlier occurrence of phenological events for all cultivars. However not all the phenological events responded to changes in the environment to the same extent and with the same level of significance. Trends of  $-0.4$ ,  $-0.7$  and  $-0.6$  days/year were detected for the phenological dates averaged over all examined cultivars for the beginning of flowering, beginning of veraison, and harvest dates, respectively. Beginning of budburst exhibited no significant trend during studied period. The trend comparison between cultivars revealed that harvest dates of early and middle ripening cultivars were advancing somewhat more than dates of late ripening cultivars.

**Key words:** grapevine, phenology, Sremski Karlovci, Serbia

### Intorduction

Recently there has been increased interest in phenology, mainly in the contest of climate change. Changes in the timing of phenological events may be one of the earliest observed plants responses to global warming. Grapevine (*Vitis vinifera* L.) is a phenologically distinct crop with the most important phenological stages being bud break, flowering, fruit set, veraison, harvest and leaf fall (Jones and Davis, 2000). The rate of development depends on grapevine cultivar, climatic conditions and geographical location. Besides climate change studies, phenology data can contribute to viticulture in different ways. They are important in determining suitable vine cultivars for a given climate regime, timely application of fertilization, pesticides, irrigation and other viticultural practices, as well as in estimating crop yields and planning harvest operations.

The earlier phenological events of grapevine have been recorded in different parts of the world (Chuine et al., 2004; Jones and Davis, 2000; Petrie and Sadras, 2008; Webb et al., 2011). Viticultural regions of Serbia have not been much studied in this regard. One of the reasons is a lack of suitably long and reliable phenological datasets, necessary for detection of meaningful trends.

This study has aimed to determine trends in grapevine phenology based on 26-year dataset for Sremski Karlovci, the wine capital of the Srem viticultural region, one of the oldest vine growing areas in Europe. The research also examined cultivar similarities/differences in phenological timing and their response to climate variability and climate change.

## Materials and methods

Phenological data were collected at the experimental station of the Novi Sad Faculty of Agriculture (45°10' N, 20°10' E, 110 m a.s.l.). The station is situated in Sremski Karlovci, 12 km away from Novi Sad on Mt. Fruška Gora's slopes. The climate is mid-latitude moderate continental (Koeppen's Cfw) with mean annual air temperature of 12.3°C and mean annual precipitation of 650 mm. Soil type is pararedzina on loess. For conducting this study, a group of 21 cultivars (Table 1), both Serbian and internationally recognized ones, was selected from ampelographic collection. The collection was established in 1979 and all cultivars were represented by 20 vines, planted with a spacing of 3 x 1 m and grown with Simple Guyot system.

Four phenological stages of grapevine were examined for the period 1986–2011:

- beginning of budburst (the date when green shoot tips became just visible);
- beginning of flowering (the date when first flower hoods were detached from the receptacle);
- beginning of veraison (the date when berries begin to develop variety-specific colour);
- harvest.

Harvest date was not precisely defined in phenological sense and mainly depended upon winery requests and some other constraints such as current weather conditions, disease outbreaks, etc.

The trends in investigated phenological events of vine were determined by the slope from a linear regression fit.

## Results and discussion

Mean dates, given as day of the year (DOY), the slope of linear regressions and corresponding correlation coefficients (R) of the investigated vine phenological stages are shown in Table 1. In the last table row, parameters' values correspond to "average cultivar". The mean date for each phenological event of "average cultivar" was obtained by averaging mean values of all examined cultivars. Time series, slopes and correlation coefficients were then determined for that "average cultivar" (Fig.1).

Table 1. Mean day of the year, slope of linear regression fit and Pearson coefficient of correlation (R) for main phenological stages of 21 vine cultivars for Sremski Karlovci, Serbia

Phenological stage	Beginning of budburst			Beginning of flowering			Beginning of veraison			Harvest		
	Mean (DOY)	Slope	R	Mean (DOY)	Slope	R	Mean (DOY)	Slope	R	Mean (DOY)	Slope	R
Portugizer	100	-0.18	0.14	148	-0.38	0.35*	200	-0.86	0.66***	256	-0.51	0.36*
Pinot noir	99	-0.40	0.29	148	-0.45	0.44*	202	-0.76	0.60***	261	-1.09	0.53**
Cabernet Sauvignon	108	-0.25	0.22	151	-0.29	0.30	212	-0.63	0.57***	271	-0.44	0.28
Gamay	98	-0.27	0.20	147	-0.48	0.47**	204	-0.57	0.51***	264	-0.59	0.35*
Merlot	104	-0.37	0.34	149	-0.44	0.42*	212	-0.69	0.50***	270	-0.58	0.33*
Probus	106	-0.19	0.17	153	-0.41	0.40*	215	-0.68	0.61***	271	-0.44	0.25
Frankovka	98	-0.16	0.12	148	-0.44	0.43*	205	-0.82	0.62***	267	-0.42	0.22
Prokupac	100	-0.26	0.18	151	-0.32	0.35*	211	-0.69	0.62***	271	-0.61	0.38*
Chardonnay	97	-0.38	0.27	146	-0.49	0.45**	205	-0.70	0.60***	254	-0.69	0.41**
Buvije	97	-0.26	0.20	148	-0.43	0.42*	194	-0.64	0.58***	249	-0.64	0.47**
Ezerjo	98	-0.12	0.10	148	-0.40	0.40*	203	-0.62	0.54**	254	-0.65	0.40*
Petra	97	-0.18	0.14	148	-0.40	0.40*	211	-0.89	0.63***	260	-0.56	0.32
Pinot Blanc	99	-0.27	0.21	147	-0.49	0.43*	208	-0.76	0.64***	257	-0.83	0.50**
Neoplanta	101	-0.26	0.19	151	-0.45	0.43*	211	-0.93	0.77***	254	-0.58	0.40*
Kreaca	101	-0.28	0.23	151	-0.43	0.42*	210	-0.81	0.60***	261	-0.67	0.44*
Muscat Ottonerl	101	-0.20	0.19	150	-0.40	0.39*	203	-0.56	0.53**	249	-0.60	0.39*
Riesling 239 20 Gm	102	-0.35	0.29	149	-0.44	0.45**	211	-0.89	0.66***	260	-0.56	0.35*

Pinot Gris	102	-0.31	0.26	147	-0.34	0.35*	204	-0.53	0.49**	255	-0.72	0.44*
Beli medenac	103	-0.18	0.15	151	-0.41	0.39*	207	-0.78	0.71***	253	-0.93	0.59**
Bagrina	103	-0.24	0.23	153	-0.52	0.45**	211	-0.77	0.61***	263	-0.73	0.39**
Riesling Italian	103	-0.23	0.23	150	-0.31	0.37*	213	-0.59	0.56**	269	-0.71	0.45**
Average	101	-0.25	0.22	149	-0.42	0.42*	207	-0.72	0.64***	260	-0.65	0.45**

\*, \*\*, \*\*\* statistical significance at the 0.05, 0.01, 0.001 levels, respectively

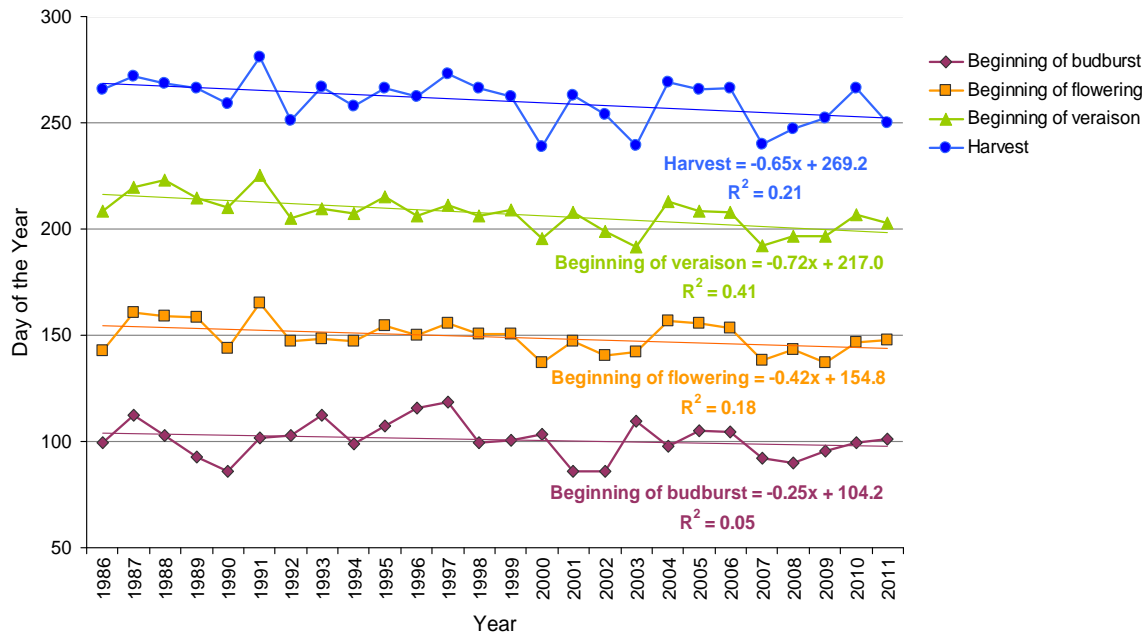


Figure 1. Time series and linear trends for the averaged phenological dates of 21 cultivars for budburst, bloom, veraison, and harvest for Sremski Karlovci, Serbia (1986–2011)

The period from the beginning of budburst to harvest averaged over all cultivars was 159 days and lasted from 10 April (101 DOY) to 17 September (260 DOY). The average time of beginning of flowering in the Sremski Karlovci region was the 29 May (149 DOY). The mean date of veraison was 26 July (207 DOY). All examined phenological stages showed a negative trend over the period of observation (Tab.1).

The steepest slope was obtained for the beginning of veraison ( $-0.7$  days/year –averaged value over all cultivars) with highest level of significance ( $p < 0.001$ ). Considering trend differences among cultivars, the highest rate of veraison date change was  $-0.9$  days/year for cultivars Neoplanta, Petra and Riesling 239 20 Gm, while smallest rate was  $-0.5$  days/year for Point Gris. The beginning of budburst exhibited no significant trend. The mean date of beginning of flowering averaged over all cultivars showed a trend of  $-0.4$  days/year ( $p < 0.01$ ). The highest observed rate of change was  $-0.5$  days/year for Bagrina, Gamay and Chardonnay and smallest  $-0.3$  days/year for Cabernet Sauvignon. The date of harvest averaged over all cultivars showed a trend of  $-0.6$  days/year ( $p < 0.05$ ). The rate of change of that phenological event varied the most between cultivars. The largest trend ( $-1.0$  days/year) was found for Pinot Noir and smallest and not significant trend ( $-0.4$  days/year) was found for Frankovka, Probus and Chardonnay).

Similar results have been found for Europe, where different phenological events occur 6–25 days earlier than 30–50 years ago (Jones, 2006). More specifically, in Germany in Lower Franconia, the phenology of grapevines has tended towards earlier occurrence during the period from 1949 to 2010 (Bock et al., 2011). Similarly as in our study, the advance in budburst was small

(1 to 2 days/decade) and not significant. The average dates for full flowering advanced 3 to 4 days/decade. Veraison showed the strongest trend in time, advancing 4 to 6 days/decade. Harvest started 2 to 5 days earlier per decade. In Italy in the productive area of Montepulciano wine, the onset of bud-break and flowering showed a negative trend whereas harvest date showed no significant variations during the period from 1970 to 2006 (Dalla Marta et al., 2010). On the other hand, observations from different viticultural regions have provided evidence of advanced harvest dates, particularly in the last 10 to 30 years. Data from Johannisberg (Rheingau, Germany) shows that the first day of harvest occurs on average of 2–3 weeks earlier than it was between the late 18th and the early 20th century (Stock et al., 2005). In Baden (southwest Germany), the average dates for the beginning of maturation of Pinot Noir had advanced by 3 weeks from 1976 to 2006 (Sigler, 2008), while in the Palatinate (Germany), harvest advanced 2 weeks (Petgen, 2007). For Chateauneuf du Pape and Tavel (southern France), harvest dates are observed 18 and 21 days earlier in comparison with the period from 1945 to 2000, respectively (Ganichot, 2002). In Alsace (eastern France), harvest takes place 2 weeks earlier (Duchêne and Schneider, 2005). In Beaune (Burgundy), harvest dates were two weeks earlier at the end of the 20th century than in the years 1960–1970 (Madelin et al., 2008).

In our study, no clear pattern of phenological timing change was detected among cultivars, except for harvest. The value of correlation coefficient between the mean harvest dates and trend values for studied cultivars was 0.33 ( $p=0.07$ ), meaning that the harvest date of late cultivars was advancing somewhat slower than harvest date of cultivars ripening earlier. To date, this trend is not significant, but if it continues in the future, it will lead to expansion of harvest period, what may have positive implications for grape processing. This finding should be taken with caution, since, as it was already stated, harvest dates are based on subjective evaluations of optimum fruit composition and depend on many constrains.

### **Conclusion**

Analysis of the 26 years of phenological data the region of Sremski Karlovci revealed that the ongoing shifts in vine phenology are toward the beginning of the year, although the observed rate of change was not the same for all phenological stages. Given that further changes to climate are likely, these observed trends in phenological timing are expecting to continue and to become even stronger. Possible consequences of advanced phenology may include shifts in regional cultivar viability and wine styles, since optimum cultivar ripening will be occurring in a warmer conditions affecting balance between sugar content and acidity, and changes in compounds development that give the aroma, colour and flavour in grapes.

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**TRENDS IN CLIMATICALLY RELEVANT TEMPERATURE INDICES FOR GRAPEVINE GROWING IN THE REGION OF SREMSKI KARLOVCI, SERBIA**

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**Abstract**

The study aimed to evaluate the structure, variability and trends of climate parameters important for grapevine growing in Sremski Karlovci for the period 1981–2007. A number of average and extreme temperature indices were calculated for annual, growing season and different growth intervals of grapevine. Results showed significant trends in: annual mean, maximum and minimum temperatures (0.06, 0.06 and 0.05°C/year, respectively); growing season mean, maximum and minimum temperatures (0.04, 0.05 and 0.05°C/year, respectively); growing degree days (10.7°C/year); annual number of days with minimum temperature higher than 90th percentile (1.0 days/year); annual number of days with maximum temperature higher than 90th percentile (0.9 days/year); the number of days with maximum temperatures above 30°C (0.7 days/year) and the number of days with maximum temperatures above 35°C (0.2 days/year); the number of tropical nights (0.6 days/year); the number of days with minimum temperatures below –2.5°C (–0.5 days/year). The most pronounced changes in climatic variables examined were recorded during the period from flowering to veraison.

**Key words:** grapevine, temperature indices, trends, Sremski Karlovci, Serbia

**Introduction**

Climate is one of the most important factors controlling grape and wine production from selection of a suitable grapevine varieties to the type and quality of wines produced (Gladstones, 1992). Recent climate change has been found to affect viticulture and wine industry across the world. Climate change is not uniform and the impact on viticulture depends on the region and the ways in which the climate change (Jones and Davis, 2000; Jones et al., 2005; Laget et al., 2008; Nemani et al., 2001; Petrie and Sadras, 2008; Ramos et al., 2008).

Out of all the climatic factors, temperature appears to be the most important, influencing the growth and development of the vine, the metabolism of acids and accumulation of sugars, the formation of different compounds that give the aroma, colour and flavour in grapes. During the winter, the completion of chilling requirements in regular and sufficient way is necessary for setting the latent buds effectively. During the spring, grapevines need sustained daily mean temperatures above 10°C to initiate growth. Growing season length and temperatures have a major influence on grape ripening and fruit quality. Jones et al. (2005) showed that average growing season temperature plays an important role for quality vintages and that increases in temperature may result in decreased quality for those varieties that are cultivated close to their optimum climatic conditions.

One of the most significant signals of the global and regional climate changes, in addition to the changes in the average temperature conditions, is the change in the extreme temperature indices. Temperature extremes during berry growth may cause premature veraison, abscission of the berries and less flavor development (Mullins et al. 1992). So-called “negative” temperatures for vine



(maximum daily temperature above 35°C) may cause partial or total inhibition of plant function, especially when associated with drought. Frost occurrence and timing affect viticulture in many ways and may present a limiting climatic factor for vine growing. Low frost risk in spring and fall, and a long frost-free period are favourable for grapevine. During the spring, temperatures below –2.5°C can adversely affect the growth and reduce bud fruitfulness, leading to lower yields and quality of grapes, while fall frosts may injure maturing canes and berries. In the winter, extreme low temperatures may cause freezing injuries to grapevines. The minimum temperature that vine may resist in the winter varies from –5 to –20 C (Winkler et al., 1974), depending on cultivar, location, characteristics of the low-temperature episode and viticultural practices.

The aim of the study was to determine recent-past trends in biophysically meaningful variables for viticulture in order to study the impacts of climatic changes and variability on grape growing in the region of Sremski Karlovci.

### **Materials and methods**

The study was done for Sremski Karlovci situated on Mt. Fruška Gora's slopes in the Srem viticultural region. Air temperature data were from climatological station maintained by the Republic Hydrometeorological Service of Serbia from 1981 until 2007. The station was located at 45°10' N and 20°10' E, 110 at an elevation of 110 m in the ampelographic collection of the Novi Sad Faculty of Agriculture, where phenological observations of a great number of grapevine cultivars have been conducting since 1986.

To analyze the climate structure in these regions, daily maximum and minimum temperatures were used to derive a number of climatically important parameters for grape growing (Table 1). Besides average values, extreme temperature indices were calculated. They were based on fixed thresholds that have biological meaning for grapevine, station-related thresholds (percentile-based indicators) and variability extremes, which give the relationship between maximum and minimum temperatures (i.e. diurnal temperature range). In addition, various periods were considered: annual, growing season and different growth intervals of grapevine.

The trends in temperature indices are determined by the slope from a linear regression fit.

### **Results and discussion**

Basic descriptive statistics, the slope of linear regressions and corresponding correlation coefficients (R) for the selected temperature indices are given in Table 1.

Growing season temperature for the period 1981–2007 averaged 17.8°C, while mean annual temperature for the same period was 12.3°C. During this 27-year period, annual mean temperature increased significantly by 0.06°C/year, maximum temperature by 0.06°C/year and minimum temperature by 0.05°C/year. Growing season mean, maximum and minimum temperatures showed significant trends of 0.04, 0.05 and 0.05°C/year, respectively (Fig. 1). Since the maximum and minimum temperatures changed nearly at the same rate, diurnal temperature range exhibit no significant trends during any period studied. Significant changes (10.7°C/year) in heat unit accumulations during the growing season (calculated as growing

**Table 1.** Descriptive and trend statistics for temperature based indices for the region of Sremski Karlovci over the period 1981–2007. **Bold** indicates significant trends at the 0.05 ( \* ), 0.01 ( \*\* ) and 0.001 ( \*\*\* ) levels

Index	Mean	SD	Max	Min	Trend (yr <sup>-1</sup> )	R	p-value
<b>ANNUAL</b>							
Average daily temperature (°C)	12.3	0.87	14.2	11.0	<b>0.05</b>	0.50	0.004**
Average maximum daily temperature (°C)	16.8	1.05	19.2	15.0	<b>0.06</b>	0.43	0.012*
Average minimum daily temperature (°C)	7.7	0.59	9.3	6.5	<b>0.05</b>	0.55	0.001***
Number of days with Tmin > 90th percentile	33.2	12.20	59.0	15.0	<b>1.05</b>	0.68	<0.001***
Number of days with Tmax > 90th percentile	36.4	15.13	75.0	17.0	<b>0.87</b>	0.45	0.009**
Number of days with Tmin < 10th percentile	36.1	11.21	59.0	19.0	-0.22	-0.16	0.219
Number of days with Tmax < 10th percentile	35.8	10.51	56.0	17.0	-0.15	-0.11	0.285
Number of days with Tmax > 25°C	88.3	16.2	123	60	0.52	0.26	0.099
Number of days with Tmax > 30°C	55.4	13.1	63	11	<b>0.74</b>	0.45	0.010**
Number of days with Tmax > 35°C	2.7	3.9	15	0	<b>0.20</b>	0.41	0.018*
Number of days with Tmin > 20°C	9.7	7.3	25.0	1.0	<b>0.56</b>	0.60	<0.001***
Number of days with Tmin < 0°C	66.7	14.8	92.0	34.0	-0.44	-0.24	0.120
Number of days with Tmin < -2.5°C	38.4	11.3	61.0	20.0	<b>-0.48</b>	-0.34	0.048*
Number of days with Tmin < -10°C	4.4	4.7	19.0	0.0	-0.10	-0.17	0.204
<b>GROWING SEASON (April – October)</b>							
Average daily temperature (°C)	17.8	0.8	19.6	16.3	<b>0.04</b>	0.40	0.021*
Average maximum daily temperature (°C)	23.3	1.1	25.9	21.4	<b>0.05</b>	0.40	0.022*
Average minimum daily temperature (°C)	12.8	0.7	14.2	11.5	<b>0.05</b>	0.59	0.022*
Diurnal temperature range (°C)	10.5	0.7	11.7	9.4	0.00	0.02	0.468
Growing Degree Days (°C)	1775.9	164.3	2156.8	1529.5	<b>10.72</b>	0.52	0.003**
<b>1 JANUARY – BEGINNING OF BUDBURST</b>							
(January – March)							
Average daily temperature (°C)	3.8	2.3	8.3	-0.5	0.07	0.25	0.100
Average maximum daily temperature (°C)	7.6	2.5	12.4	3.0	0.08	0.27	0.088
Average minimum daily temperature (°C)	0.1	2.1	4.3	-4.0	0.06	0.24	0.119
Number of days with Tmin < 0°C	42.4	16.0	67.0	9.0	-0.35	-0.18	0.200
Number of days with Tmin < -2.5°C	26.8	13.3	54.0	0.0	-0.48	-0.29	0.078
Number of days with Tmin < -10°C	3.3	4.9	19.0	0.0	-0.12	-0.19	0.176
<b>BEGINNING OF BUDBURST – BEGINNING OF FLOWERING (April – May)</b>							
Average daily temperature (°C)	15.0	1.4	17.7	11.6	<b>0.06</b>	0.36	0.031*
Average maximum daily temperature (°C)	20.1	1.7	23.5	16.0	<b>0.07</b>	0.34	0.042*
Average minimum daily temperature (°C)	9.8	1.1	11.9	7.3	<b>0.05</b>	0.39	0.024*
Number of days with Tmin < 0°C	0.7	1.2	5.0	0.0	0.00	0.01	0.472
<b>BEGINNING OF FLOWERING – BEGINNING OF VERAISON (June – July)</b>							
Average daily temperature (°C)	21.4	1.2	24.1	19.1	<b>0.10</b>	0.66	<0.001***
Average maximum daily temperature (°C)	26.8	1.5	30.5	24.4	<b>0.11</b>	0.60	<0.001***
Average minimum daily temperature (°C)	15.9	1.1	18.2	13.6	<b>0.09</b>	0.68	<0.001***
Number of days with Tmax > 25°C	39.0	7.2	55.0	26.0	<b>0.46</b>	0.51	0.003**
Number of days with Tmax > 30°C	15.4	7.5	31.0	4.0	<b>0.57</b>	0.61	<0.001***
Number of days with Tmax > 35°C	1.2	2.4	11.0	0.0	<b>0.16</b>	0.52	0.003**
Number of days with Tmin > 20°C	5.7	4.4	14.0	1.0	<b>0.40</b>	0.72	<0.001***
Diurnal temperature range (°C)	10.9	0.8	12.9	9.4	0.02	0.19	0.169
<b>BEGINNING OF VERAISON – HARVEST</b>							
Average daily temperature (°C)	20.2	1.1	22.9	17.5	0.01	0.08	0.347
Average maximum daily temperature (°C)	25.6	1.5	29.3	21.5	-0.01	-0.05	0.407
Average minimum daily temperature (°C)	14.8	0.9	16.9	13.4	0.03	0.28	0.081
Number of days with Tmax > 25°C	34.2	7.4	51.0	20.0	-0.14	-0.15	0.222
Number of days with Tmax > 30°C	11.5	7.3	27.0	1.0	0.03	0.03	0.432
Number of days with Tmax > 35°C	1.5	2.4	10.0	0.0	0.04	0.15	0.229
Number of days with Tmin > 20°C	3.8	4.2	16.0	0.0	0.16	0.31	0.060
Diurnal temperature range (°C)	10.8	1.1	12.8	8.1	-0.04	-0.30	0.067

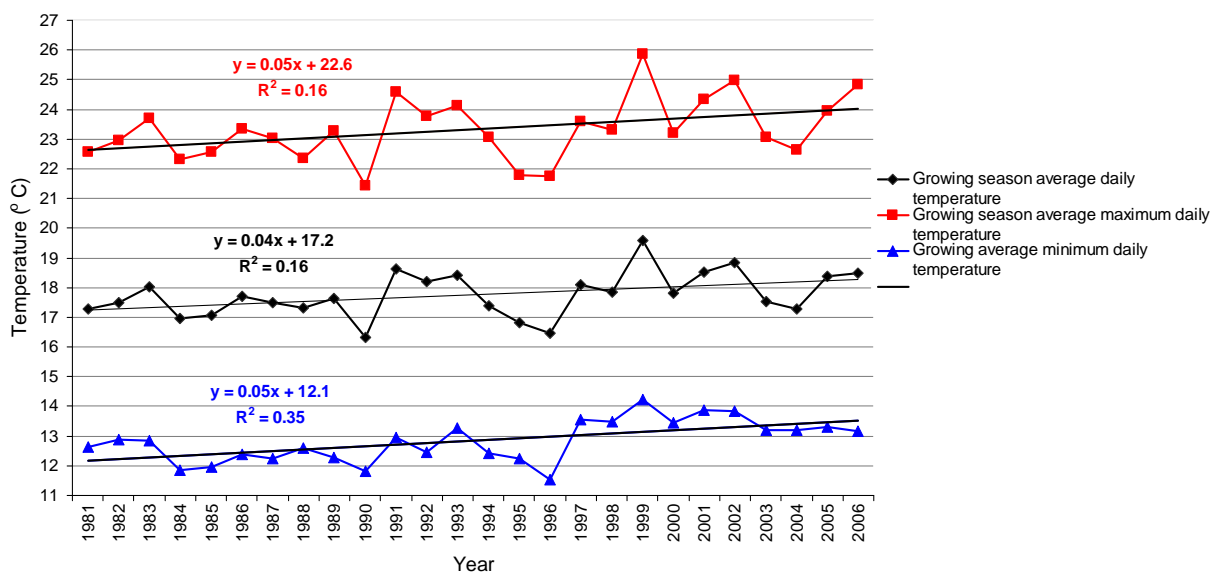


Figure 1. Time series and linear trends for growing season average, maximum and minimum daily temperatures from 1981 to 2007 for Sremski Karlovci, Serbia

degree-days using a base of 10°C with no upper cut-off) were detected. Results showed a positive trend in the annual series of most indices related to high temperature. Trends of extreme indices based on percentiles, warm days ( $T_{max} > 90$ th percentile) and warm nights ( $T_{min} > 90$ th percentile) were significant. The annual number of tropical days ( $T_{max} > 30$  °C) has increased at a faster rate than that of the annual number of summer days ( $T_{max} > 25$  °C). The trend of number of days with so-called “negative” temperatures for vine ( $T_{max} > 35$ °C) also showed significant positive trends, as well as the number of tropical nights ( $T_{min} > 20$ °C). Examining annual and seasonal frequencies of days with low temperatures, a decline in the number of days with minimum temperatures below 0, -2.5, -10°C was found. The trend was negative, but not significant, except for the annual number of days with  $T_{min} < -2.5$ °C.

For the period from beginning of flowering to beginning of veraison, the strongest and most significant trends were detected for all selected indices. Conversely, the same indices did not show significant trends during ripening. This is a consequence of fact that September temperatures showed even negative trend over the study period, while June and July temperatures showed the significant positive trends (data not shown).

## Conclusion

Analysis of temperature based indices for Sremski Karlovci, revealed a general warming in this viticultural region over the period from 1981 to 2007. It was found that the daily maximum temperature is getting more extreme, whereas the minimum temperature is getting less extreme. The strongest increase was detected for hot related extremes such as summer days and tropical nights. The nature and trends of climate variables and bioclimatic indices important for grapevine growing suggest that grapevine is and will be grown in warmer conditions than before. Consequently, vine phenology has already been affected and earlier dates of flowering, veraison and harvest have been observed for a number of varieties in the region of Sremski Karlovci (Ruml et al., 2013). The next step in the research will be to relate trends in phenology to trend in climatic variables and to identify temperature indices that have the greatest impact on vine growth and production.

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## DETERMINATION OF RADIOCAESIUM IN BLUEBERRIES

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### Abstract

In forest ecosystems, radionuclides are deposited in surface organic layers of the trees and other plants like grass, berries moss and lichen. From artificial radionuclides,  $^{137}\text{Cs}$  is one of the most important fission product and it is of particular concern in the natural environment due to a long half life ( $T_{1/2} = 30$  years), easy migration in the tropic chains and great bioavailability. Because of that, this artificial radionuclide is presents in environment especially in food samples, even 20 years after Chernobyl accident.

This paper presents the results of measurement the activity concentration of  $^{137}\text{Cs}$  in blueberries (42 samples) for the period August 2011 to December 2012, which were imported in Serbia from different countries. Measurements were performed in Radiation and Environmental Protection Department in the Vin a Institute of Nuclear Sciences. Concentrations of  $^{137}\text{Cs}$  were determined by gamma spectrometry using a HPGe detector.

The obtained results show that the activity concentration of  $^{137}\text{Cs}$  in blueberries ranged from MDC (minimum detectable concentration) to  $404 \text{ Bq kg}^{-1}$ . Recommended level of activity concentration for  $^{137}\text{Cs}$  in blueberries in Serbia is  $150 \text{ Bq kg}^{-1}$  (Official Gazette of the Republic of Serbia, 2011). Out of tested samples, 79 % of blueberries met the defined criteria of radiological safety.

Based on the obtained results for activity concentration of  $^{137}\text{Cs}$ , the annual effective dose due to ingestion of blueberries for adults was calculated.

**Keywords:** Radiocaesium, blueberries, gamma spectrometry, annual effective dose.

### Introduction

Radiation and Environmental Protection Department in the Vin a Institute of Nuclear Sciences performs regular control of radioactivity of imported goods (foodstuffs, fertilizers, building materials, items of general use, etc). In Serbia, based on the regulation (Official Gazette of the Republic of Serbia, 2011) limit values of activity concentrations of certain radionuclides are defined for different materials (drinking water, foodstuffs, feeding stuffs, drugs, items of general use, building materials and other goods to be placed on the market). Among others, for imported blueberries and other berries which are subject to control, limit value for the activity concentration of  $^{137}\text{Cs}$  is  $150 \text{ Bq kg}^{-1}$ .

Blueberries are among the most popular fruits for home and market gardening. Today, blueberries are grown commercially also in South America, Australia, New Zealand, Asia, South Africa and Europe (Strik, 2005; Wach, 2008). Almost 10% of the world total blueberries cultivated area is located in Europe where Poland and Germany are more important producers (Delian et al., 2010). Blueberries require acid soils, and a soil pH between 4.5 and 5.2 is ideal. However, blueberries can be productive on places where the pH is as high as 6.0 (Strik, 2006). Adequate soil for the growth of blueberry is sour and moderately moist. Blueberries are growing in the area of deciduous and beech forests.

$^{137}\text{Cs}$ , which can be found in blueberries, is a fission product of concern due to its long physical half life of 30.2 years and its high bioavailability.  $^{137}\text{Cs}$  is water soluble and can rapidly

enter the biological cycles and accumulate in terrestrial ecosystems, behaving in a very similar way to potassium. As a result, it is mainly deposited in soil and vegetation (Bourcier et al., 2010).

The atmospheric nuclear weapon tests carried out in the 1950's and 1960's and up to 1980 led to a global contamination with fallout of radionuclides, and in particular of  $^{137}\text{Cs}$ , especially in the northern hemisphere. Additional contamination in Europe occurred after the Chernobyl accident in 1986 (Bourcier et al., 2010).  $^{137}\text{Cs}$  is produced during fission of nuclear fuel, as a result of nuclear weapons testing, operation of nuclear reactors, reprocessing of spent fuel and reactor accidents. This causes radionuclide release into the environment which can enter the human food chain. An additional small risk does exist from production of radioisotope sources for medical and industrial uses (Carpenter et al., 1995).

Forests are effective trapping systems for fallout radionuclides, which persist in the forest ecosystem for much longer than in cultivated agricultural land. In general, the consumption of forest products may contribute to the increase the internal human dose, which is caused by intake of radionuclides via ingestion. For example, blueberries and mushrooms are dominant and they are commonly consumed (Vaaramaa et al., 2009).

After deposition of fallout and interception by the above ground parts of the plant, radionuclides in soluble form can be retained, absorbed and translocated to fruits as well as to other parts of the plant. Such processes are important not only for short term releases, but also play a role during the following years in perennials, especially (re)translocation within the plant, but also along with the process of resuspension. Contamination of fruits following wet or dry deposition can be directly on the exposed fruit surface, or initially on any of the exposed plant surfaces followed by absorption and translocation to fruit (Carini and Bengtsson, 2001).

One of the factors in the assessment of risk to human health, is the risk of ionizing radiation which can be monitored via the annual effective dose. In assessing the annual effective dose which the population received, one of the elements is the dose which is received via ingestion.

The aim of this study was to calculate the annual effective dose which the population can received due to ingestion of 150 g fruits per day (IAEA, 1999), if we assume that all of ingested fruit are blueberries.

### Materials and methods

The samples of blueberries imported from different countries were investigated. The preparation of samples included weighing and placing into the 500 cm<sup>3</sup> Marinelli beakers (IAEA, 1989). Since the purpose of measurement is screening, the counting of the samples was conducted immediately after preparation, without drying. The total of 42 samples of blueberries for the period of 17 months were analyzed.

Samples were measured using a high purity germanium detector (HPGe) with relative efficiencies of 18 % and 20 % and energy resolution of 1.8 keV for the 1332 keV  $^{60}\text{Co}$  peak. Calibration of detectors for measurement of food samples was performed using of silicone resin matrix in geometry of the plastic Marinelli beaker of 500 cm<sup>3</sup>, (Czech Metrological Institute, Praha, 9031-OL-208/08, type ERX) spiked with a series of radionuclides ( $^{241}\text{Am}$ ,  $^{109}\text{Cd}$ ,  $^{139}\text{Ce}$ ,  $^{57}\text{Co}$ ,  $^{60}\text{Co}$ ,  $^{203}\text{Hg}$ ,  $^{88}\text{Y}$ ,  $^{113}\text{Sn}$ ,  $^{85}\text{Sr}$  i  $^{137}\text{Cs}$ ) with total activity of 40.624 kBq on the day April 15, 2008.

Counting time interval was 3600 s. The spectra were analyzed using the program GENIE 2000. The activity of  $^{137}\text{Cs}$  was determined from its 661 keV –energy.

The accuracy and reproducibility of gamma spectrometry systems were verified on a periodic basis every week. Total background count rate without a source is monitored to verify that the detector and shield have not been contaminated by radioactive materials. Energy calibration is checked in whole region before applying usual quality control (QC) procedure for gamma spectrometry measurement. The total activity of calibration source is used to check the efficiency calibration and the general operating parameters of the gamma spectrometry system (source positioning, contamination, library values, and energy calibration). The detector shield

background, detector efficiency, peak shape, and peak drift are measured and verified if they are within the warning and acceptance limits. For that purpose  $^{60}\text{Co}$  and  $^{133}\text{Ba}$  sources were used.

The activity concentration of  $^{137}\text{Cs}$  in the samples was calculated using the equation:

$$A = \frac{N}{t \times P_x \times E_f \times m} \quad (1)$$

where  $N$  is count of the sample corrected on background,  $t$  counting time (s),  $P$  probability of gamma decay (%),  $E_f$  full energy peak efficiency (%),  $m$  the mass (kg) of the sample.

Minimum detectable concentration (MDC) was calculated by the equation (2):

$$MDC = \frac{LLD}{t \times P_x \times E_f \times m} \quad (2)$$

where  $LLD$  is the detection limit,  $LLD = 2.71 + 4.65\sqrt{B}$ , where  $B$  is count of the background.

The combined measurement uncertainty of the results was calculated at the 95% level of confidence ( $k = 2$ ).

A possible risk of radioactivity for human health is expressed by the individual annual effective dose due to ingestion ( $E_{ing}$ ). A contribution to the individual annual effective dose to an adult from blueberry consumption may be calculated, according to (IAEA, 2001) using equation (3):

$$E_{ing} = H \times A \times DF_{ing} \quad (3)$$

where  $E_{ing}$  is the individual annual effective dose due to ingestion (Sv),  $H$  the annual intake of blueberries (kg per person) which in our case is 54.75 kg,  $A$  the activity concentration of  $^{137}\text{Cs}$  ( $\text{Bq kg}^{-1}$ ) and  $DF_{ing}$  the dose conversion factor for ingestion of  $^{137}\text{Cs}$  defined as the dose received by an adult per unit intake of radioactivity and its value is  $1.3 \times 10^{-8} \text{ Sv Bq}^{-1}$  (IAEA 1999, Kala 2001).

## Results and discussion

The results of measurements of  $^{137}\text{Cs}$  in blueberries, which were imported in Serbia from different countries are presented in *Table 1*. The activity concentration of  $^{137}\text{Cs}$  in  $\text{Bq kg}^{-1}$  ranged between MDC and 404. Recommended level of activity concentration for  $^{137}\text{Cs}$  in Serbia is  $150 \text{ Bq kg}^{-1}$  (Official Gazette of the Republic of Serbia, 2011). Based on the results presented in *Table 1*, it can be seen that out of measured samples, 79 % of blueberries met the defined criteria of radiological safety, while 21 % of the samples do not met the criteria. As can be seen from *Table 1*, all the samples which do not met the criteria for radiological safety were imported from Ukraine.

Also, based on the obtained results for the activity concentration of  $^{137}\text{Cs}$ , the annual effective dose for adults was calculated (assuming that adults eat 150 g of fruit per day (IAEA, 1999), in this case blueberries) and presented in *Table 1*. The annual effective dose values due to the ingestion of  $^{137}\text{Cs}$  from blueberries were ranged from 0.9 to  $287.5 \mu\text{Sv}$ . The values of the annual effective dose due to ingestion blueberries for all samples that met the criteria of radiological safety are below the recommended reference level of  $100 \mu\text{Sv}$  (Official Gazette of the Republic of Serbia, 2011), so they are not dangerous to human health.

**Table 1.** The activity concentration of  $^{137}\text{Cs}$  in blueberries and the annual effective dose for age group ( $> 17$ ).

	Country of import	$^{137}\text{Cs}$ ( $\text{Bq kg}^{-1}$ )	Annual effective dose ( $\mu\text{Sv}$ )
1.	Republic of Macedonia	$< 1$	/
2.	Montenegro	$< 1$	/
3.	Republic of Macedonia	$< 1$	/
4.	Republic of Macedonia	$< 2$	/
5.	Republic of Macedonia	$< 2$	/

6.	Russia	$3.8 \pm 1.8$	2.7
7.	Republic of Macedonia	$3.5 \pm 1.3$	2.5
8.	Bosnia and Herzegovina	< 2	/
9.	Republic of Macedonia	< 2	/
10.	Ukraine	$112 \pm 12$	79.7
11.	Ukraine	$83 \pm 9$	59.1
12.	Russia	< 1.3	/
13.	Ukraine	$276 \pm 25$	196.4
14.	Ukraine	$46 \pm 6$	32.7
15.	Ukraine	$60 \pm 7$	42.7
16.	Republic of Macedonia	$1.3 \pm 0.2$	0.9
17.	Ukraine	$121 \pm 12$	86.1
18.	Ukraine	$71 \pm 8$	50.5
19.	Ukraine	$160 \pm 15$	113.9
20.	Republic of Macedonia	< 1.4	/
21.	Russia	$3.3 \pm 1.0$	2.3
22.	Republic of Macedonia	< 1.5	/
23.	Ukraine	$5 \pm 2$	3.6
24.	Russia	$2.1 \pm 1.0$	1.5
25.	Republic of Macedonia	< 1	/
26.	Montenegro	< 1	/
27.	Republic of Macedonia	$2.0 \pm 0.7$	1.4
28.	Montenegro	$7.1 \pm 2.0$	5.1
29.	Ukraine	$206 \pm 19$	146.6
30.	Ukraine	$20 \pm 3$	14.2
31.	Ukraine	$93 \pm 10$	66.2
32.	Republic of Macedonia	< 1	/
33.	Ukraine	$266 \pm 24$	189.3
34.	Ukraine	$325 \pm 30$	231.3
35.	Montenegro	$2.4 \pm 1.1$	1.7
36.	Ukraine	$71 \pm 7$	50.5
37.	Ukraine	$63 \pm 7$	44.8
38.	Ukraine	$404 \pm 36$	287.5
39.	Ukraine	$395 \pm 34$	281.1
40.	Ukraine	$230 \pm 21$	163.7
41.	Ukraine	$264 \pm 23$	187.9
42.	Montenegro	$5 \pm 2$	3.6

### Conclusion

42 samples of blueberries imported from different countries in Serbia, were investigated in order to determine the activity concentration of  $^{137}\text{Cs}$  in them. The obtained results for the activity concentration of  $^{137}\text{Cs}$  ranged between MDC and  $404 \text{ Bq kg}^{-1}$  and the obtained values for the annual effective dose ranged between  $0.9\text{--}287.5 \mu\text{Sv}$ .

Considering the fact that 21 % samples of blueberries expressed higher values of the activity concentration of  $^{137}\text{Cs}$ , as well as the annual effective dose than the recommended limit value, there is a need for regular control blueberries which are imported.

All samples which do not met the criteria for radiological safety were imported from Ukraine and apparently this is consequence of the nuclear accident at Chernobyl.



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## **DRAINAGE IMPACT ON STRUCTURAL COMPOSITION PSEUDOGLEY SOILS IN REPUBLIC OF SERBIA**

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### **Abstract**

Pseudogley soils (as per WBR, 2006 classification, Stagnosol) cover significant agricultural areas within the Republic of Serbia. This type of soils are of great importance for agricultural production due to their potential fertility, as well as the fact that occur mainly on flat areas suitable for the application of mechanized agriculture. Many years of intensive crop production lead to changes that reduce soil's productive capacity and lead to structural changes in the soil composition. The aim of this study was to examine the extent to which arable farming affect changes in the structure of pseudogley soils in drained and un-drained plots. The study was conducted in the experimental drainage field at Varna Institute of Soil Science, ten years after the installation of drainage systems. The soil waterlogging on the part of the experimental field, in which the control is derived horizontal pipe drainage led to elevated processes of structure deterioration, the formation of crumbly aggregates, as well as the decrease in water stability of structural aggregates.

**Key words:** soil structure, soil aggregates, drainage, pseudogley

### **Introduction**

Throughout the territory of west and northwest Serbia pseudogley soils cover an area of about 360,000 ha or 20 % of the total area, whereas 285,000 ha or 15.7 % of the area is covered only by pseudogley features (urovic et al., 2010). The largest pseudogley complexes are located in the pre-Pannonian part of northwestern Serbia, on flat terraces and hills formed by abrasive action of the Pannonian Sea. In the west and northwest area of Serbia, pseudogley is non-calcareous, the soil acidification is high, which is confirmed by pH values of active and exchangeable acidity. The soils, especially the surface horizons, are characterised by a high reduction of bases in the adsorption complex (the degree of base saturation of the adsorption complex is low, and declines up to 17 %). Also, the greatest amount of fine substances and organic particles, distinguished by high adsorption capacity, are leached from the surface horizons. Therefore, in west and northwest Serbia, pseudogley is a poorly humose soil. In natural conditions, pseudogley structure in the surface horizon is fine-crumby, and at the depth greater than 50cm the structure becomes polyhedral prismatic (Tanasijevic et al., 1966).

### **Materials and Methods**

The investigation was conducted at experimental drainage field of the Institute of Soil Science, Varna, (44°41'38" N; 19°39'10" E) located on the tenth kilometer road which leads from Šabac, southeast to Loznica and Valjevo, at the entrance to the village Varna (44°41'38" N; 19°39'10" E) (Pivic, 2013). Ten years ago permanent drainage systems have been installed in the area. The aim of

this study was to examine the extent to which arable farming affect changes in the structure of pseudogley on drained and un-drained plots.

The structural composition and stability of soil aggregates are most important characteristics of soil fertility. From the agronomical aspect, the most significant are structural aggregates from 1 to 10 mm (Edwards, 1991; Amezketta, 1999). The most significant properties for the evaluation of soil structure are the content of macro-aggregates, their mechanical strength and water stability, as well as porosity (Sorochkin, 1991). Soil structure is a dynamic value that depends on the soil properties, climate and tillage conditions (Angers, 1998). The factors affecting soil structure and aggregate composition are tillage, irrigation and climate (Guerif et al., 2001). Soil aggregate stability declines rapidly as the consequence of cropping, and the diameter of dry aggregates increases (Kandeler and Murer, 1993; Shepherd et al., 2001).

On the experimental drainage field of the Institute of Soil Science in Varna in 1978 a drainage sample plot was established. Drainage sample plot consists of two separate parts of rectangular form, separated by a road for mechanization. One part consists of three plots: A, B, C, and the other part consists of six plots designated I to VI. All the plots are of the same size 75.0x52.0 m, individual area 0.39 ha. The basis for dewatering of the nine sample plots is flexible perforated PVC drainage pipes, spacing 25 meters. Within the plots there are two drains Ø80 mm, at the depth of 0.95 m. Drain length is equal to plot length and amounts to 52 m, minimal design slope is 0.25 %. The experiment was amended in 2002 by adding two additional variants of drain spacing treatments: 20 m (field A) and 30 m (field C), at the same depth of 0.90 m, and perforated PVC pipes Ø80 mm.

Exploration and other field study including profile digging and description were performed in 2012 (profiles 1-4). Samples were taken in autumn after harvesting. The samples were taken from genetic horizons in the disturbed and undisturbed state. Mechanical composition was determined by pipette method, the samples were prepared with sodium pyrophosphate. Macro-aggregate analysis of the soil was performed by dry sieving method (Shein et al., 2001). The samples (about 2 kg) were sieved on a series of screen mesh sizes 10, 5, 3, 2, 1, 0.5 and 0.25 mm. The sieved samples from each sieve were weighed and their percentage in the sample was calculated.

Water stability of structural aggregates was determined using the Savinov's wet sieving method (Shein et al., 2001), i.e. by sieving in still water through a nested column of sieves consisting proportionally of all aggregate fractions separated in the dry structural analysis. Sieving was performed on screen mesh sizes of 3, 2, 1, 0.5 and 0.25 mm. After sieving and drying the samples at 105 °C, the samples were weighed and their percentage in the sample was calculated. Structure coefficient ( $K_s$ ) was calculated by the expression:

$$K_s = \frac{\text{Content of agronomical most favourable aggregates, 0.25-10 mm}}{\text{Total content of the aggregates } <0.25 \text{ mm} + >10 \text{ mm} \text{ separated by dry sieving}}$$

Aggregate composition of the soil was evaluated by the following scale (Shein et al., 2001):

- >1.5 - good aggregate composition,
- 1.5-0.67 - satisfactory aggregate composition,
- <0.67 - unsatisfactory aggregate composition.

The soil structure was evaluated according to Dolgov and Bakhtin's scale (cit. Gajic, 2006).

## Results and Discussion

The processed data of the soil mechanical analysis (Table 1) show that soil texture, determined using the USDA triangle (Soil Survey Manual, 1955), is loam (I) to clay loam (GI). The data on the soil mechanical composition show that the soil to the depth of 50 cm is loam, with physical clay contents in 61.6 and 69.1% respectively. The percentage of clay increases with profile depth, so in deeper horizons, the soil becomes clay loam. The increase of silt and clay contents with depth corresponds to the values characteristic of pseudogley.

Table 1. Particle size analysis of the soil

Profile	Horizon	Depth (cm)	Coarse sand >0.2 mm	Fine sand 0.2-0.02 mm	Silt 0.02-0.002 mm	Clay <0.002 mm	Total sand >0.02 mm	Silt+clay <0.02 mm	Soil textural class	Colour
PROFILE 1 CONTROL	Aoh	0-25	4.8	30.2	42.7	22.3	35.0	65.0	I	10YR7/3;6/3
	Eg	25-45	4.3	28.6	43.9	23.2	32.9	67.1	I	10YR7/3;6/3
	Btg	45-75	3.8	28.9	35.2	32.1	32.7	67.3	GI	10YR4/2;3/2
	Btg	>75	1.0	31.2	34.8	33.0	32.2	67.8	GI	10YR4/2;3/2
PROFILE 2 DRAINAGE 25 m	Aoh	0-30	2.6	35.8	38.1	23.5	38.4	61.6	I	10YR7/3;6/3
	Eg	30-47	2.7	28.0	45.8	23.5	30.7	69.3	I	10YR7/3;6/3
	Btg	47-72	1.8	27.2	37.7	33.3	29.0	71.0	GI	10YR4/2;3/2
	Btg	72-105	0.6	27.2	42.2	30.0	27.8	72.2	GI	10YR4/2;3/2
PROFILE 3 DRAINAGE 20 m	Aoh	0-30	2.0	29.2	43.8	25.0	31.2	68.8	I	10YR7/3;6/3
	Eg	30-54	3.6	28.3	42.6	25.5	31.9	68.1	I	10YR7/3;6/3
	Btg	54-80	1.9	26.6	35.9	35.6	28.5	71.5	GI	10YR4/2;3/2
	Btg	80-105	2.0	25.7	36.6	35.7	27.7	72.3	GI	10YR4/2;3/2
PROFILE 4 DRAINAGE 30 m	Aoh	0-23	3.4	27.5	44.1	25.0	30.9	69.1	I	10YR7/3;6/3
	Eg	23-40	3.6	29.4	44.8	22.2	33.0	67.0	I	10YR7/3;6/3
	Btg	40-64	2.2	24.4	40.8	32.6	26.6	73.4	GI	10YR4/2;3/2
	Btg	64-100	1.4	22.9	36.9	38.8	24.3	75.7	GI	10YR4/2;3/2

Soil structure is a dynamic characteristic which depends on numerous factors of soil genesis, physical and chemical properties, land use, and application of agro-technical measures. After ten years of intensive crop production, there were significant changes in pseudogley structure. The most represented fraction on all variants is agronomical the most favourable aggregate fraction (10-0.25 mm). The data on aggregate percentage separated by dry sieving show the decrease in the percentage of agronomical favourable aggregates from 76.9 % drained surfaces to 12.4 % the free surface drainage. The percentage of coarser aggregates (>10 mm) is higher on the land without drainage, which points to deteriorated conditions that lead to the formation of crumbly structure. The percentage of granular aggregate (5-10 mm) is somewhat higher in the drained plots, whereas the percentages of all the separated aggregate fractions of smaller dimensions are higher in the non-drained variant. Based on the structure coefficient (Figure 3), all three drained variants are within well-structured soils, although the structure coefficient of the soil in control variant is higher (3.33; 2.62; 2.19).

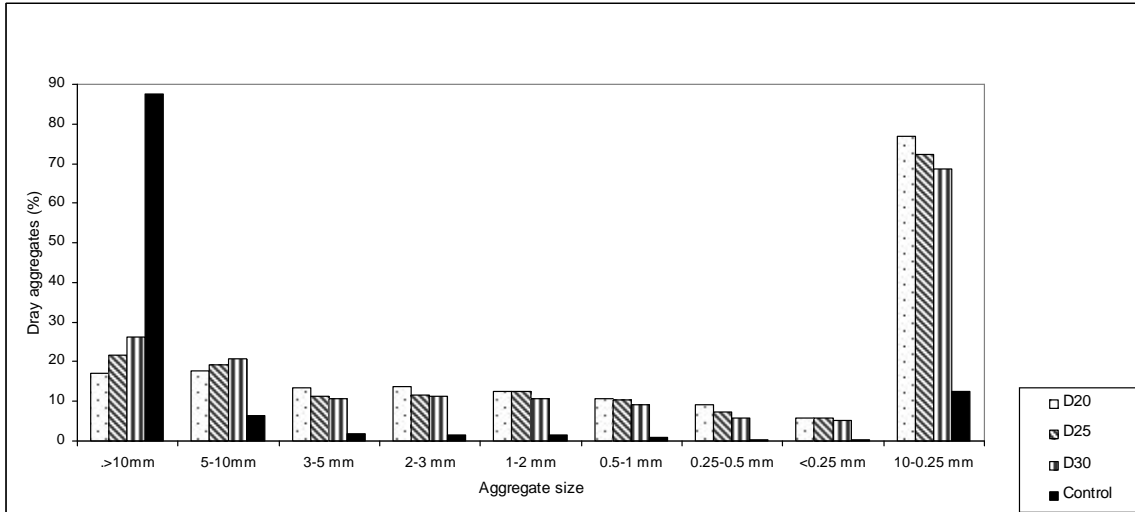


Figure 1. Dry aggregate size distribution

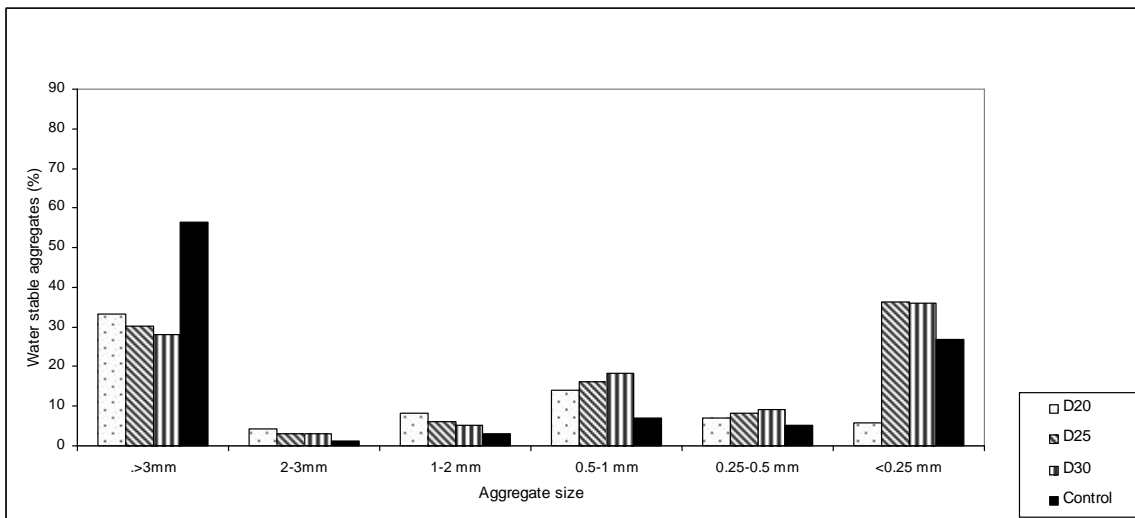


Figure 2. Water stable aggregates

The pseudogley structure in the exceedingly waterlogged areas was highly deteriorated. The percentage of agronomical favourable aggregates decreased to only 12.4 %, whereas the sizes of the dominant (87.5 %) aggregates in the soil were large, which is very unfavourable for agricultural production. The high percentage of macro-aggregates points to a significant deterioration of the conditions, which leads to the formation of crumby structure. Aggregates smaller than 0.25 mm practically was absent and the percentage of aggregates between 0.25 and 3 mm were insignificant. Based on the structure coefficient, which is only 0.14, it can be concluded that the soil structure is unsatisfactory, and that the soil waterlogging in the conditions of insufficient soil drainage, leads to rapid deterioration of soil structure which is one of the most essential indicators of the soil properties and soil fertility.

The evaluation of soil structure, based on the percentages of the agronomical most favourable aggregates, according to Dolgov and Bakhtin’s scale, shows that all four variants are characterised by a good structural state. The waterlogged soil structure, based on the content of 0.25-10 mm air-dry aggregates, is evaluated as poor.

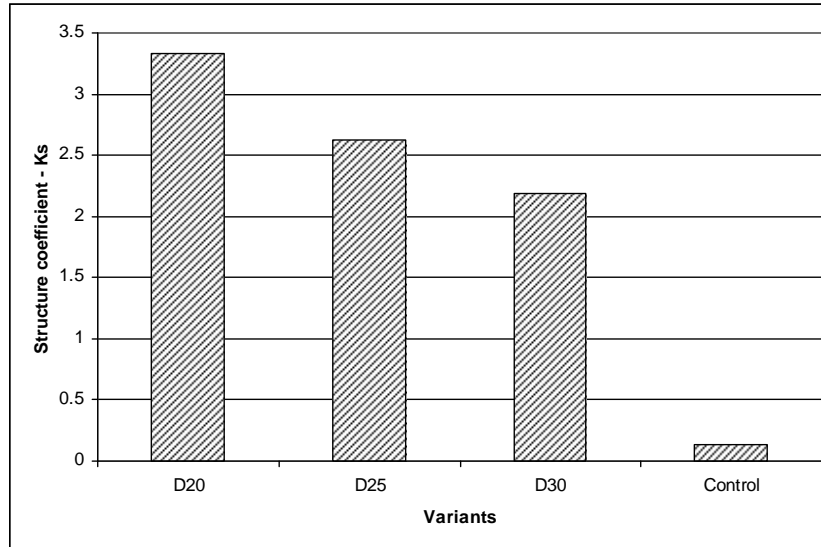


Figure 3. Structure coefficient (Ks)

The wet sieving analysis shows that the aggregate resistance to decomposition in water is higher on the variant with smaller drainage spacing. From total 48.3 % in dry sieving aggregates larger than 3 mm, on the variant of drain spacing 20 m, resistance to decomposition in water is 33.3 %, for the variant of drain spacing 25 m, from total of 52.3 %, the resistance to decomposition in water is 30.1 %, while for the variant of drain spacing of 30 m, from total of 57.6 % in dry sieving aggregates larger than 3 mm, resistance to decomposition in water is 28 %. For the control plot, without drainage on flooded surface, from total of 95.6 % in dry sieving aggregates larger than 3 mm, resistance to decomposition in water is 56.5 %.

The wet sieving analysis also shows that, in the non-drained variants, there was a decrease in the percentage of water-stable aggregates larger than 1mm, and it's amounting up to 60.8 %. Conversely, the percentage of the fraction of water-stable aggregates smaller than 1 mm was higher in the control variant - 39.2 %, which indicates that the ratio of these fractions in the drained and non-drained variants has changed compared to the content of aggregates separated by dry sieving. The above changed ratio also refers to the fractions smaller than 0.25 mm, where on the control variant it amounts to 27 %, while on the variants with drainage it is in the range between 33.2 % to 36.4 %. The variety exposed to permanent waterlogging, control variant, shows that only the percentage of aggregates larger than 3 mm is higher than in the variants with drainage, which is not unexpected as in dry sieving the percentage of these aggregates in the waterlogged variety is almost 97.1 %, and the dominant aggregates in that fraction are crumbly aggregates. The contents of all other fractions of water-stable aggregates in the waterlogged soil are lower.

Based on the above results, it can be concluded that the pipe drainage system leads to some changes in the structural composition of pseudogley, primarily in the sense of a mild increase in the percentage of larger aggregates (>5 mm) and also their greater liability to decomposition in water (decrease in the percentage of water-stable aggregates >1 mm).

### Conclusions

The soil type of the study site in the experimental drainage field of the Institute of Soil Science in Varna is pseudogley. The surface horizon is loam and the deeper horizons are clay loam. The soil structure in drained plots is good, with structure coefficient greater than 2. On the structure

coefficient is low and it amounts 0.14. The ten-year-long implementation of drainage system caused some changes in the soil structural composition in the sense of a mild increase in the content of larger-sized aggregates (>5 mm), but also their greater liability to decomposition, as well as a mild decrease in the percentage of water-stable aggregates larger than 1 mm. The soil waterlogging led to elevated processes of structure deterioration, the formation of crumbly aggregates, as well as to the decrease in water stability of structural aggregates.

### Acknowledgment

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## ECONOMIC EVALUATION OF BIOMASS AS A SOURCE OF ENERGY IN VOJVODINA

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### Abstract

World trends in the area of utilization of renewable sources of energy indicate that all developed countries are rapidly orienting towards intensive utilization of the renewable sources of energy. The fact that our country has relatively high rate of growth of energy consumption (6 – 7% annually) and that our reserves of primary energy are six times weaker in relation to the world's average, strongly directs us to rational utilization of even the smallest volumes of disposable fuels. According to the way in which the biomass has been utilized so far in Vojvodina, it was estimated that 30 - 50% of the total biomass volumes can be used for energy purposes. During the previous period, the biggest part of this potential was being burned, other part was used as a floor covering in livestock production and insignificant part was ploughed in. Utilization for other purposes is quite rare. This potential of the biomass can be exploited for the production of thermal and electric energy, as well as for the insulation in civil engineering, and other purposes as well.

**Key words:** biomass, crop production, potentials

### Introduction

Using renewable energy as an alternative to conventional fuels (oil, coal, wood) is a target pursued by our country and the EU countries, where the “work paper on renewable energy” was adopted, on January 16th 2007, by the Brussels United Union Board, which stated that 20% rise in renewable energy production inside the UE energy sector by 2020. In conformity with the CE Legislation and HG 1844/2005, “biomass is a biodegradable fraction of agricultural waste and residue products, forestry domain and the similar industries, as well as the biodegradable fraction from the municipal and industrial waste”. According to some estimation, the total annual production of biomass in Vojvodina is 10.75 million tons or 30 - 40% less, as compared to earlier periods (Brkic, Janic, 2010). Agriculture, forestry, wood industry and communal business produce large quantities of biomass, and/or organic matter, production residues or waste. Those quantities are approximately the same each year. Even 30 years ago, it was estimated that 9 million tons of biomass was produced by agricultural industry of Vojvodina only (Brkic at all, 1979). Since one part of this quantity of the organic matter can be utilized as an alternative fuel or as a raw material for production of fuel, it is also called renewable source of energy (or abb. RSE). Research activities of other authors (Zekic, Tica, 2010) are also in conformity with previous quotations. The potentials of crop residues of the basic field cultures is over 5,5 million tons in Vojvodina, annually, and/or over 9 million tons in Serbia. Regardless the significant potential we should have on mind that only one part of biomass can be utilized in energy purposes or some other purposes. Namely, in order to preserve the fertility of the soil, crop residues should be ploughed in, which increases the level of humus in soil and preserves its fertility. Much better way to preserve the soil is to use crop residues as a floor covering on animal farms. In this way, the produced dung can be used for soil preservation. Development of the energy crisis stimulates the opinion that great volumes of the biomass can be utilized for combustion and the production of thermal and electric energy. Namely, it is well known that significant volumes of biomass are needlessly burnt on fields, because it is



difficult to plough it in. In order to plough the biomass in, it is necessary to cut it up previously, which requires additional energy. Besides, livestock fund in Serbia was significantly reduced during the previous period, the technology of animal breeding was changed, and necessary floor coverings for animals were minimized. Due to the aforesaid, it is possible to use only 30 - 50% of the total estimated volumes of crop residues for the production of thermal and electric energy, each year.

### Material and Method

Information about the types and volumes of the biomass was collected from the statistical records for most important agricultural crops. Specific features of the performed estimations required the use of technical literature and practical experience. Because the official statistics do not make records of the volumes of crop residues and other side-products, grain mass versus straw mass ratio had to be defined (Brkic, Janic, 1998, 2000; Alimpic, 1983). The obtained mass ratio relates balance (or storage) degree of humidity in certain products. The total potential was calculated into energy-equivalent volumes of lignite. Economic estimation of the aforesaid potential was executed in accordance with the market price of lignite in Serbia.

### Results and discussion

Calculation presented in the following table provides the data relating average annual production of the biomass in Vojvodina, relating the period of 5 years (2003 - 2007). If this data is compared to previously executed research activities it is possible to conclude that the quantity of biomass originating from crop production in Vojvodina was reduced for 32,3%. Estimation of the annual potential is 12,6 million tons of biomass in Serbia, whereas nowadays, only 9,97 million of tons are being produced. In Vojvodina, this drop is even more intensive. The total production used to be around 9 million tons, whereas today it is 6.1 million of tones (Brkic, Janic, 2010), and only 5 million of tones of biomass for the observed crops.

This change is also noticeable in the very types of biomass. The production of hop is insignificant, domestic seed products and oilseed rapes are gradually being reduced. The production of wheat is halved, and the quantity of corn is three times lower. The following table presents the calculation of the available crop residues, equivalent volumes of coal and estimated values of this potential.

Table 1. Potentials of the biomass in crop production in Vojvodina

No	Culture	Mass of grain (t/ha)	Mass of relations (t/t)	Mass of straw (t/god)	Energy-equivalent quantity of coal (t/god)	Estimated value (€)
1	Wheat	1.062.949	1:1	1.062.949	982.864	20.640.140
2	Corn	2.283.398	1:1	2.283.398	2.189.560	45.980.754
3	Corn cob	0	1:0,2	456.680	437.912	9.196.151
4	Sunflower	337.361	1:2	674.722	716.314	15.042.603
5	Shell	0	1:0,3	101.208	107.447	2.256.391
6	Soybean	260.550	1:2	521.100	560.361	11.767.580
	<b>Total</b>	<b>3.944.258</b>		<b>5.100.057</b>	<b>4.994.458</b>	<b>104.883.619</b>

Establishment of energy-equivalent quantities of coal is based on minimal energy value of lignite, which is 14.6 MJ/kg<sup>17</sup>. This value is relatively high, considering the fact that the thermal value of raw lignite from the Kovin mine is roughly 10,54 MJ/kg<sup>18</sup>. Energy values of some types of crop residues were calculated in conformity with the previously performed research activities (Zubac,

<sup>17</sup> <http://hypertextbook.com/facts/Energy Density of Coal.htm>

<sup>18</sup> Energy balance of the Autonomous Province of Vojvodina - Plan for 2011. year

Bubalo, 1995) although, in real exploitation conditions, these values are generally lower. The total energy value of side-products of crop production is 1,741 M toe, which makes 59,25% of the total final production of energy in AP Vojvodina, according to the plan for the year 2011.

Calculation of the economic value was made on the basis of the price of lignite in Serbia. In all that, it included the minimal price of raw coal, and which is 21 €/t, on average. If we take into consideration previously quoted utilization of this potential, which is 30%, it is possible to talk about the potential whose value goes to 31 million € annually, where its utilization could satisfy 17,7% of the final energy consumption in AP Vojvodina.

### **Conclusion**

According to the research, it was concluded that in Vojvodina, more than 5 million tons of crop residues are produced each year, all originating from crop production (the rest is grain production). It is estimated that this quantity of biomass can be utilized for the production of thermal energy and electric energy, in the percentage of 30 – 50 %. The rest could be directed to increase the soil fertility, it could be used in livestock breeding, vegetable production, insulation in civil engineering, fodder cropping, and etc. Tendencies of trends in production of certain crops indicate to reduction in share of wheat and grains, in the seeding structure. It is assumed that this trend will continue in the following period as well, and/or that the crop production will reach production substitution of grains with industrial crops. The assumption of further spreading of the areas seeded with industrial crops comes out of the expected improvement of the economic position of the agriculture, and in accordance with that, increased investments into production which should result in preferring of the capitally intensified cultivations, for example, cultivations utilized in industrial production. With further development of biological potentials of cultivations, the real assumption is that available quantities of crop residues will be reduced. Namely, it is obvious that as the sorts are being improved, and/or as new sorts and hybrids are being cultivated, the ratio straw-grain will be changing in favour of grain. In future, this tendency will reduce available quantities of side-products, in crop production. On the other hand, if we analyse the quality of available volumes of the biomass, we must, first of all, take care of humidity of straw of certain cultivations, at harvest time. Namely, straw from wheat and other corns, at harvest time, has 15 - 20% of humidity. The quoted value is appropriate for energetic application. This circumstance makes the straw from grains the basic potential source of energy in analyzed sector. As different to the straw from grains, the research indicates that the cornstalk in October, and/or at harvest time, has up to 48% of humidity. This makes cornstalk inconvenient for baling, so its preparation is done in the form of stacks. Although our practice includes mechanization for mechanical preparation of the cornstalk, this way of preparation is very extensive and is not convenient for large energetic exploitation. The quoted statement relates production of commodities, which is organized on large properties. In case of small individual sectors, due to latent unemployment and low level of commodity production, exploitation of crop residues has more perspective. The same frames and contributors refer to crop residues of the sunflower. It does not include the sunflower coat, which, as a potential source of energy and the very technology of sunflower processing, is concentrated in oil plants. In this way, the total issue of its utilization is brought down to adaptation of combustion boilers or the process of briquetting, which make this, almost conventional energy substance, the matter of market sales. Besides, we should not forget the possibility of production of the biomass for combustion, as the main cultivation on less productive soils.

### **Acknowledgement**

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## INTERACTION OF BENZYLAMINOPURINE (BAP) AND INDOL BUTIRIC ACID (IBA) ON ROOT INDUCTION IN OLEA EUROPEA L.

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### Abstract

Olive var. “*Kokërr Madhi i Beratit*” is an important table variety for Albania despite major difficulties with respect to vegetative propagation from leafy stem cuttings. Leafy stem cuttings were obtained from 1-year-old olive shoots sampled on 25 April 2012 during the growing season. The shoots were collected at the same height of tree crown to avoid the effect of juvenility on root induction. To improve the rooting of olive cuttings, different concentrations of BAP (6-Benzyl aminopurine), 100ppm, 150ppm, 200ppm and 250ppm were tested in combination with IBA (Indol Butiric Acid) 4000ppm. After treatments the stem cuttings were planted in greenhouse equipped with an automatic mist system. Fifty days after the beginning of rooting treatments, cuttings were scored for the presence of callus, percentage of rooted cuttings, root number per cutting and root length. BAP inhibits adventitious root formation, but adding it to IBA in a small ratio (1:30-1:40) improved the rooting. The combination of IBA 4000ppm + 100ppm (40:1) and IBA 4000ppm+150ppm BAP modified significantly higher rooting of cuttings. Those combinations of growth stimulators induce also a higher number of roots per cutting in comparison with those treated with IBA alone.

**Key words:** olive, root, shoot, Benzylaminopurine, Indol Butyric Acid

### Introduction

The propagation of the olive up to now has been realized by various ways, such as seeds, hardwood cuttings, leafy stem cuttings, spheroblasts, etc. Propagation with leafy stem cuttings under mist system is the most accepted method in the world. Olive, var. “*Kokërr Madhi i Beratit*” expose major difficulties with respect to vegetative propagation from leafy stem cuttings. This experiment aimed at increasing the rooting ability of olive cuttings of this variety using IBA with different concentrations of BAP. GA<sub>3</sub> and BAP inhibits adventitious root formation, but in some cases stimulate it (7,6.8).

Auxins play a central role during lateral root development (1). Also, has been reported that cytokinins are important endogenous regulators of lateral root initiation (4). Cytokinins and auxins have been implicated in the regulation of root development, being the principal players (3). Cytokinins regulate root meristem activity via modulation of the polar auxin transport. Auxins and cytokinins modulate the root meristem size, but they differ in concentration range at which they can act either in a stimulatory or an inhibitory mode (3). The phytohormones auxins and cytokinins are important regulators of the developmental fate of pluripotent plant cells (5). Cytokinins influence cell-to-cell auxin transport by modification of expression of several auxin transport components and thus modulate auxin distribution important for regulation of activity and size of the root meristem (3). The study reported in this paper was carried out to examine any possible interaction between IBA and BAP which might influence the rooting activity of stem cuttings in *Olea europea*, cv. “*Kokërr Madhi i Beratit*”.

## Materials and Method

Leafy stem cuttings (15-20 cm long each) from one year old olive shoots of “Kokërr Madhi i Beratit”, grown in Levan village (Fieri district) were conducted to examine the influence of the combination IBA with BAP on the root initiation. The shoots were sampled in spring (25 April) during the 2012 growing season. Leafy stem cuttings with four leaves were prepared in the morning and their bases were dipped for 7 sec in solution of 4,000 ppm IBA, the appropriate concentration on rooting of olive stems, in combination with different treatment of BAP as follows:

### Experiment one:

- 1<sup>st</sup> treatment 0ppm IBA
- 2<sup>nd</sup> treatment 2000ppm IBA
- 3<sup>rd</sup> treatment 4000ppm IBA
- 4<sup>th</sup> treatment 6000ppm IBA
- 5<sup>th</sup> treatment 10000ppm IBA

### Experiment two:

- 1<sup>st</sup> treatment 4000ppm IBA+0BAP
- 2<sup>nd</sup> treatment 4000ppm IBA+100ppm BAP
- 3<sup>rd</sup> treatment 4000ppm IBA+150ppm BAP
- 4<sup>th</sup> treatment 4000ppm IBA+200ppm BAP
- 5<sup>th</sup> treatment 4000ppm IBA+250ppm BAP

After treatments, the stem cuttings were planted in greenhouse equipped with an automatic moist system. The layout of experimental design was completely randomized with 4 replications of 100 cuttings per treatment. Cuttings were evaluated 50 days after planting for percentage of rooted cuttings, primary root number and mean primary root length. Differences between means of each treatment were analyzed by the Duncan multiple range test ( $P < 0.05$ ).

## Results and discussion

As it can be seen in Table 1 the olive var. Koker Madhi i Beratit has major difficulties with respect to vegetative propagation from leafy stem cuttings. The percentage of rooting without IBA and with different concentrations of IBA was very low (6,75% maximum). The percentage of rooting was higher in the treatment of 4000ppm IBA, but the difference was not significant however, we supposed the concentration of 4000ppm IBA as the best, and we used this concentration of IBA in combination with different concentrations of BAP to see the interaction of these two hormones on the root induction of this olive variety.

**Table 1.** Effect of IBA on rooting of “Kokerr Madhi i Beratit” olive cuttings

Treatments/Replications	R1	R2	R3	R4	Means
0% IBA	0	2	1	4	1,75a
2000ppm IBA	4	11	1	7	5,75a
4000ppm IBA	5	6	7	9	6,75a
6000ppm IBA	0	1	8	3	3,00a
10000ppm IBA	5	2	0	1	2,00a

The number (percentage) of rooted cuttings, using of IBA alone and in combination with BAP on rooting of “Kokerr Madhi i Beratit” olive cuttings are summarized in Table 2. In determining the optimum of benzylaminopurine (BAP) requirements for rooting, the best result was obtained when 4000ppm IBA (control) was combined with 100ppm and 150ppm BAP (respectively, 22% and 24,75 higher than the control). The percentage of rooting was decreased considerably by increasing the BAP concentration to 200 and 250ppm. This means that auxins and cytokinins modulate the root meristem size, but they differ in concentration range at which they can act either in a stimulatory or an inhibitory mode (3). The promotion of BAP on rooting appears to be due to stimulation of juvenility in tissues of cuttings, or perhaps cytokinin regulates root meristem activity via modulation of the polar auxin transport (3). Also, may be, BAP increased the sprouting buds, and consequently, the synthesis of any rooting cofactor. The stimulation of rooting by combination of IBA with BAP reported in this paper is shows that the stem cuttings of olive var, Kokërr Madhi i Beratit has little or has not at all BAP.

To promote root induction of leafy stem cuttings of olive var. “Kokërr Madhi i Beratit” must used as root induction hormones the combination of auxins and cytokinins at appropriate concentration

**Table 2.** Effect of IBA alone and in combination with BAP on rooting of “Kokërr Madhi i Beratit” olive cuttings

Variante/perseritje	R1	R2	R3	R4	Mean
4000ppmIBA+0BAP	6	7	9	8	7,5a
4000ppmIBA+100ppmBAP	49	36	15	18	29,5b
4000ppmIBA+150ppmBAP	38	16	45	30	32,25b
4000ppmIBA+200ppmBAP	16	9	21	11	14,25c
4000ppmIBA+250ppmBAP	25	3	29	2	14,75c

\* Separation by Duncan’s multiple range test, at  $P < 0.05$ , +Mean of three replications

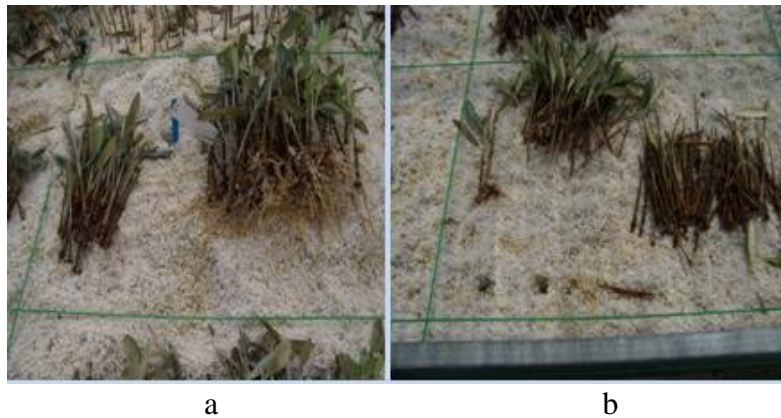
### Conclusion

The stimulation of rooting by combination of IBA with BAP reported in this paper is shows that the stem cuttings of olive var. Kokërr madhi i Beratit has little or has not at all cytokinins.

To promote root induction of leafy stem cuttings of olive var. “Kokërr Madhi i Beratit” must used as root induction hormones the combination of auxins and cytokinins at appropriate concentration.



**Figure-1.** Cuttings planted in mist propagation (40 days after planting) Left (IBA+BAP). Right (IBA)



**Figure-2** Rooted cuttings(50 days after planting

a- (IBA+BAP) b-(IBA)

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## QUANTITATIVE EVALUATION OF ERODED SEDIMENTS IN THE UPPER IBRAHIM RIVER WATERSHED, LEBANON

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### Abstract

Soil erosion is one of the major problems facing the mountainous agricultural lands in Lebanon. Sediments carried in the river water are a criterion of pollution. In order to determine the quantity and acting factors of soil erosion, a study was conducted in the Upper watershed of Ibrahim river. Thus, seven localities were investigated on the two major streams feeding the river, located between 808 m and 1488 m in altitude. These localities are connected to sub-basins representing 88.9% of the river watershed (312.7 km<sup>2</sup>). Water samples were collected during the spring season in April, May and June 2013. Suspended sediments were determined by decantation. Simultaneously bed load samples were taken in order to determine their texture as well as their mineral composition. Water flow and sediments load were the highest in April, in all sub-basins. Sediments load reached 713.72 mg L<sup>-1</sup> and 298.60 mg L<sup>-1</sup> for the localities 2 and 3 in April and decreased to 61.77 mg L<sup>-1</sup> and 25.94 mg L<sup>-1</sup> in May. The monthly eroded soil reached 704 tons during April in location 2 and 662 tons in location 6. Within each sub-basin the land cover, slope length and gradient influenced water sediments load. Where orchards are predominant, such as in the upper watershed, high soil erosion was found. The sub-basin predominated by grassland (52.64% of area) and stable bare rocks (45.11% of area) generated lower sediments load.

**Key words:** Ibrahim river, snow melting, erosion, land cover, sediments.

### Introduction

Lebanon climate is generally Mediterranean with hot dry summers and cold rainy winters. A small and mountainous country, Lebanon presents a complex geomorphology and several agro-ecological zones caused by different agro-climatic conditions and complex orography. The Mount-Lebanon zone extends from the North to the South hills with a total length of 160 km. Mount-Lebanon zone includes a large number of catchments which vary in size. Most of these coastal rivers are fed by rain, snowmelt and karstic springs. The hydrological regime of these rivers is characterized by high flows in winter caused by intense precipitation as well as a spreading of these flows during the spring supported by snowmelt. Lebanon enjoys abundant ground and surface water, but the supply of potable water has been reduced by pollution caused by population growth, industrial development and expansion of irrigated agricultural land (El-Fadel et al., 2000). The unregulated exploitation and overpumping of private wells have also impaired aquifer quality (Jurdi, 1992; El-Moujabber et al., 2006; Darwish et al., 2011).

In agricultural lands, fertilizers increase the soil contamination with nitrogen and phosphorus. These may appear in the stream after a heavy rain due to soil erosion and runoff water. Soil erosion is one of the major causes of land degradation in Lebanon (BouKheir, 2006). Soil erosion is critical in Lebanon due to the steep topography, the lack of wide coastal plain and to the absence of the protective land cover and inappropriate land, forestry and watershed management. This study focuses on Ibrahim river catchment with a basin area of 326 km<sup>2</sup> and a slope gradient of 55 to 60 m km<sup>-1</sup>. The river length is 44 km and presents an annual discharge of 328 Mm<sup>3</sup>. This study was



conducted in the upper watershed of the river during snow melting with the objectives of characterizing the sediments carried in the water during three spring months.

### Materials and method

#### Ibrahim river watershed

The Ibrahim river basin is influenced by Mediterranean climatic conditions especially temperature and precipitation. The precipitation ranges between 900 mm and 1400 mm with an average of 1108 mm. The land cover in the watershed plays an important role in controlling the erosion processes. It is one of several important factors affecting the infiltration rate and runoff water. Dominated by bare soil (123.9 km<sup>2</sup>), then by woodland (120.3 km<sup>2</sup>), agricultural land occupies 8% of the watershed area.

#### Sampling and analysis

The study was done in seven sites in the upper stream (Table 1). Samples were collected during three monthly visits: 17 April, 11 May and 16 June 2012. Water flow in sites 1, 2 and 3 was not permanent.

Table 1. Coordinates, the altitude and the main textural class of the soils found in the seven sampling locations.

	Longitude	Latitude	Altitude (m)	Textural class
1	34° 7'34.72"	35°53'45.63"	1488	Clay Loam
2	34° 7'33.78"	35°53'50.68"	1457	Clay Loam
3	34° 7'32.26"	35°53'54.05"	1438	Clay Loam
4	34° 7'20.71"	35°54'29.01"	1324	Loam
5	34° 6'32.12"	35°54'28.41"	1250	Clay Loam
6	34° 6'28.42"	35°53'23.37"	1119	Clay Loam
7	34° 5'19.28"	35°51'49.93"	808	Clay loam

Water samples collected in a 5000 mL plastic gallon, were subjected to decantation for 2 weeks. Then the clear water was pumped with a manual pump. The decantation was repeated till a volume of 20 mL was achieved, then the samples were dried in an oven at 105°C. The water flow during each monthly visit was obtained empirically, by determining the speed of a moving object and the cross-section of the site. The water flow and the suspended sediments allowed to calculate the monthly amounts of sediments. Bed load samples were dried in the oven at 45°C. Then a sub-fraction (200 g) was passed on a plastic sieve (63 µm). The fractions larger than 63 µm were passed through three sieves 2000 µm, 250 µm and 180 µm. Then the 5 fractions were weighed.

#### Soil data

Soil data were retrieved from the Soil and Terrain database (SOTER), based on the new soil map of Lebanon at 1:50,000 scale (Darwish et al., 2006). SOTER is a relational database system that provides complete information on the soil i.e., location, landform, lithology, slope gradient, drainage conditions, erosion, surface stoniness, texture, rootable depth, diagnostic horizons and other physical and chemical properties (Darwish et al., 2000).

## Results and discussion

### Land cover and soil properties

The area of the sub-basins varied between 1.4 hectare for sub-basin 3 and 18388.1 hectares for sub-basin 7 (Table 2). Land use of the sub-basin feeding each location was established. Agricultural lands were predominant in sub-basins 1, 2, 3 and 6.

Of the soil properties affecting the erosion, soil texture and organic matter content play an important role. The textural classes of the soils surrounding the sampling sites were mainly clay loam (Table 1). The sand fraction varies between 29.94% and 39.37%, the clay content between 23.28% and 38.26%, and the silt from 21.81% to 36.49%. Soil organic matter has an effect on soil aggregation which in turn influences soil water infiltration, moisture content and drainage. Lebanese soils are poor in organic matter (Darwish et al, 2009) with a limited ability to aggregate. Soils with high clay and organic matter contents increase the potential for aggregate formation that lead more to soil stability and prevent erosion (Rice, 2002). Thus the studied sub-basins are more susceptible to erosion due to low soil organic matter.

Table 2. Land cover in the sub-basins feeding the 7 studied locations in the watershed of Ibrahim river.

Sub-basin	Land cover (% of area)						Area (ha)
	Buildings	Agricultural land	Woodland	Grassland	Bare soil and rocks	Lake	
1	0.00	71.25	0.00	21.55	7.06	0.14	82.9
2	0.00	54.22	7.94	13.66	21.48	2.70	225.2
3	0.00	60.95	0.30	38.752	0.00	0.00	1.4
4	0.00	0.63	1.62	52.64	45.11	0.00	8618.6
5	0.00	0.00	99.19	0.00	0.81	0.00	21.2
6	< 1%	60.73	12.78	20.92	3.75	1.34	471.6
7	< 1%	5.84	8.79	49.25	35.46	0.11	18388.1

### Slope

Soil particles can be detached and transported from the soil surface, especially when located on steep land. Increased run-off occurs on heavier soils when soil structure is poor and the soil has lost its ability to soak up melting snow. This run-off may be sufficient to scour and detach fine soil particles leading to discoloured run-off (also known as soil wash). However, it does not always cause rill formation. Any improper cultural practices, or compaction caused by these operations will increase surface run-off and erosion risks. At the higher altitudes of each sub-basin, ranged between 1715 m and 2573 m (Table 3), the energy of rain splashing is absent in the period from April to June, due to the snow cover. Soil freezing causes the deterioration of aggregates leading to the transport of fine soil (clay particles) by runoff water (Ollesch et al., 2005). This could explain why even soils with high clay contents are subject to erosion. Low levels of clay and the combination of several factors could explain the high level of sediments in the sub basin 2. A similar range of clay was recorded for the other sites.

The intensity of erosion caused by snow melt is determined by the rate of melting, the amount of water equivalent, soil permeability, disintegration of soil aggregates caused by frost, moisture in the soil and vegetation. Melting rates are usually much smaller than the intensity of rain but frozen soil in the surface layer may reduce the infiltration rate. Repeated freezing and thawing of soil plays a considerable role in the degradation of soil erosion by water. During the process of snow melt combination of intense rainfall, frozen soil and saturated soil surface leads to erosion and development of serrated ravines. Not taking into consideration the sub-basin 3 because of its small area (1.4 ha) and sub-basin 5 fed by groundwater (*Roueiss* spring), the slope of the sub-basin 2 was the highest (20.7 degrees) with land use occupied by agricultural lands (71.25%). These factors explain the high load of sediments in location 2. The effect of the slope meets the conclusion obtained in a study on terraced orchards in two Greek islands, where soil erosion increased significantly when the slope gradient reached 25% (Koulouri and Giourgo, 2007).

Table 3. Higher and lower points of each sub-basin feeding the 7 sampling locations in the upper watershed of Ibrahim river.

Sub-basin	Length (km)	Lower point H min (m)	Higher point H max (m)	Slope (%)
1	2.64	1488	2063	15.7
2	2.74	1457	2090	20.7
3	0.34	1438	1715	30.4
4	16.54	1324	2573	15.2
5	0.70	1250	1868	35.5
6	4.80	1119	2038	17.5
7	19.48	808	2573	15.2

#### Sediments in the Upper Ibrahim river watershed

Sediments are the largest water pollutant that affects water quality physically, chemically and biologically. In this study the sub-basin 3 showed a high level of erosion per unit area (11.61 tons ha<sup>-1</sup>) followed by sub-basins 2 and 6 (Table 4). Because of its very small area, the sub-basin 3 will be excluded from the discussion. The level of sediments in April 2013 was higher than those during May and June 2012 despite the higher temperatures intensifying snow melt due probably to soil warming and improved recharge. Locations 1, 2, 3 and 6 were alimented by runoff water due to the melting of the snow at this period. Location 4 was alimented by a huge watershed, whereas location 5 was directly alimented by *Roueiss* spring. Finally location 7 was at the convergence of the two springs *Afqa* and *Roueiss* (Table 4).

Table 4. Monthly flow, concentration of suspended sediments and sediments lost per unit area of each sampling location during April, May and June 2012.

	Location	Flow (m <sup>3</sup> )	Sediments (mg L <sup>-1</sup> )	Sediments (tons month <sup>-1</sup> )	Sediments (tons ha <sup>-1</sup> )
April	1	620524.8	88.87	55.1	0.67
	2	987033.6	713.72	704.5	3.13
	3	54432.0	298.60	16.3	11.61
	4	6270566.4	9.64	60.5	0.01
	5	64180000.0	2.66	170.8	8.06
	6	4149273.6	159.59	662.2	1.40

	7	Not accessible			
May	1	74995.2	21.81	1.6	0.02
	2	404974.1	61.77	25.0	0.11
	3	13499.1	25.94	0.4	0.25
	4	742452.5	21.74	16.1	0.00
	5	45356000.0	2.27	103.0	4.86
	6	1967017.0	11.65	22.9	0.05
	7	91854000.0	8.8	808.3	0.04
June	1	Dry			
	2				
	3				
	4	20736.0	0.00	0.1	0.00
	5	19168000.0	0.89	18.9	0.89
	6	298598.4	0.00	0.6	0.00
	7	39779000.0	0.01	194.0	0.01

The main variables affecting water erosion are precipitation and surface runoff. The impact of raindrops striking the soil surface can detach the soil particles, break down soil aggregates and disperse the aggregate material causing splash erosion. Experiments with artificial rainfall showed that sediment concentrations tended to increase not only with the slope gradient but also with slope length, particularly when side-slope gradients exceeded 10%. The effect may be the result of a change from erosion dominated by raindrop detachment and raindrop-induced flow transport to erosion dominated by raindrop and flow transport (Kinnell, 2000).

#### Bed load sediments

Bed load sediments were separated into 4 classes according to their size (Table 5). For the locations 2 and 3 the percentages of the fraction below 63  $\mu\text{m}$  were higher than the other locations. Usually the fraction smaller than 63  $\mu\text{m}$  is transported in water as total suspended solid but some of it will deposit on the bottom of the stream as the velocity of water decreases. The quality of the particles found in the bed load reflects to a large extent the type of soils surrounding the watershed. The range of sand in the region surrounding the sites 1, 2, 3, 4, and 5 was between 16 and 47% whereas for the locations 6 and 7 it increased to 64 and 82%. The high occurrence of sand in surrounding soils was reflected in the values of sand in the bed load sediments 80.9% in the location 6 and 78.25% in the location 7. Location 5 was dominated by gravel (65% of sediments) reflecting the origin of this water as ground water. The bed load textural classes showed that in locations 1 and 6 no change was noticed with time, indicating the homogeneity of eroded soil in these sub-basins. The absence of homogeneity in the other locations could be linked to changes in the water velocity during snow melting leading to erosion deposition and migration of soil particles along the river.

The bed load samples were subjected to several tests in order to determine the concentration of available nitrate, phosphorus, potassium and the percentage of organic matter. The available nitrate ranges from 4.9 to 18.8  $\text{mg kg}^{-1}$ , from 1.4 to 8.6  $\text{mg kg}^{-1}$  for Olsen phosphorus, from 40 to 250  $\text{mg kg}^{-1}$  for exchangeable potassium and from 3 to 15  $\text{g kg}^{-1}$  for organic matter (Table 5). Nitrates are easily leached from the soil with high rainfall. All the samples were in a range between low to medium except for the location 1 that showed a high level of nitrate compared to the other locations. Olsen test estimates the plant-available phosphorus. All samples showed a low level of

available phosphorus illustrating the low mobility of phosphorus in soil. Available potassium ranged between low to medium levels in the sediments samples. The K concentrations found in the locations 1, 2, 3 and 6 suggest a significant contribution from heavily fertilized agricultural land.

Table 5. Concentrations of minerals in the bed load sediments sampled in April, May and June 2012.

Location	Date	Concentration (mg kg <sup>-1</sup> )			Organic matter (g kg <sup>-1</sup> )
		Nitrate	Olsen-P	K	
1	April	18.8	8.6	250	15
	May	13.4	7.6	170	12
2	April	6.7	2.7	90	5
	May	5.4	5.2	150	5
3	April	5.8	1.4	150	5
	May	5.8	5.1	140	5
4	May	13.8	2.8	20	12
6	April	7.1	3.7	80	7
	May	6.7	3.6	70	5
	June	4.9	5.1	120	5
7	May	4.9	3.9	50	3
	June	11.2	3.9	40	7

### Conclusion

This study was conducted to evaluate the quantity of eroded soil in the upper watershed of Ibrahim river during snow melting. Water load sediments tended to be favoured by the snow melting that increased water flow. The total quantity of lost soil was the highest in location 7, which was fed by all studied sub-basins, during May 2012 (808 tons), followed by location 2 in April 2012 (704 tons) then by location 6 during the same month (662 tons). An important quantity of sediments was delivered in the river with some 1669 tons from the first 6 locations in April. This quantity of sediments will significantly contribute to the degradation of agricultural land.

Data revealed that the high level of suspended sediments in water was influenced by the land cover especially agricultural land dominated by orchards. Their occurrence on steep land, in the absence of cover crop and under poor agricultural practices, lead to high levels of eroded soils. This value reached 11.61 tons ha<sup>-1</sup> in location 3. In sub-basin 4 low levels of sediments were generated because of the grassland (52.64% of sub-basin area) acting as ground cover, on one side, and the bare rocky ground (45.11% of the area) on the other. Sub-basins dominated by orchard delivered a huge quantity of sediments in the river. Action must be taken in order to preserve this productive soil by improving the knowledge of the farmer for the application of better agricultural practices as cover crops, mulching and reduce the frequency of ploughing. Further studies must be done during the autumn season with the first rains in order to obtain the full water load sediments.

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## SOIL EROSION OF THE CUVERAK RIVER BASIN (WEST SERBIA)

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### Abstract

Various factors of erosion, natural and anthropogenic, and general conditions of the area of Cuverak torrential flow contribute to the understanding of the intensity of basin soil erosion. Midterm amount of erosion sediments is  $W_{\text{year}} 646.05 \text{ m}^3/\text{year}$ . The value of the specific total annual erosion sediments at the mouth of the Cuverak in Kamenica ( $G_{\text{yr/sp}}$ ), is  $171.48 \text{ m}^3/\text{km}^2/\text{year}$ .

**Key words:** soil, erosion, river basin, sediment production

### Introduction

Soil is the basis of agricultural production, and thus important for the survival of the human race. Formation of soil is a continuous process, but parallel, there are processes of soil degradation. Process of soil regeneration is very slow. The effects of different factors of erosion change the soil and geological substrate. Changes in soil can be slow or fast, as a result erosion characteristics are slow or fast. In Serbia more than 90% of the total soil area is affected by erosion of various types and intensity (Djorovic and Kadovic, 1997). In the Republic of Serbia, it is registered that each year from an area of 21,000 ha layer of soil depth of 16.0 cm has been removed. (Spalevic, 1997). In the Republic of Serbia (Central Serbia) there is 1.221 million ha of eroded soil and 36,000 ha is steady now (Statisti ki godišnjak, 2008).

In the region of a ak trends of increase in temperature and decrease in rainfall are evident (Šekularac, 2002). That climatic changes cause deterioration of the physical characteristics of the soil, increasing its erodibility, reducing the role of protective vegetation, and aggravated its natural and artificial regeneration. All this affects intensification of the process of erosion, both surface and deep forms. Negative impact of the erosion endangers agriculture, forestry and water management, thus there is an increasing need for erosion protection and soil reclamation.

Quantitative amount of erosion and regarding how much sediment it causes, is shown on the part of the river basin Kamenica (part of the West Morava river basin), its sub-basin, the area of which is its right tributary of the first order, Cuverak.

### Materials and methods

Using the ground of reconnaissance, the elements of the configuration of the river basin were monitored and shown. This basic method is complemented by the use of topographic, geologic and soil maps of certain scales, allowing for defining the nature and impact of natural erosion agents in the studied basin. Using the method of rainfall interpolation by rain-gradient (Bonacci, 1984) and calculation of air temperature for any altitude (Dukic, 1984), meteorological parameters

were calculated for the basin. Quantitative indicators of soil erosion were calculated using the Erosion Potential Method (Gavrilovic, 1972).

### Results and discussion

The basic elements of the river basin, important for the occurrence of soil erosion, are size, length, volume and its shape. The river basin Cuverak encompasses an area of 1.62 km<sup>2</sup>, the natural length of the main watercourse, L, is 2.2 km, circumference, C, is 6.5 km. In the river basin Cuverak the maximum elongated hydrographic are underdeveloped upper and lower stream. In these parts regularly no major tributaries. In the middle is the main source of sediments. The presented basic elements of the Cuverak river basin and the special features of its relief, the geological substrate, the distribution of land, and the land use have contributed that the process of erosion of the studied river basin has specific quantitative indicators. The main parameters of the Cuverak river basin relief, agents which have the primary responsibility for the occurrence of erosion, are shown in the Table 1.

The mean sea level ( $M_{sl}$ ) of Cuverak basin is 646.34 m (Table 1), which is calculated by the method of the separation of contour lines every 100 m in height.

The mean elevation difference of the Cuverak river basin, D, is 261.34 m (Table 1).

The average river basin decline,  $I_m$ , is 29.6% (Table 1). The condition of the relief of an area is indicated by the coefficient of basin relief erosion energy ( $E_r$ ), m/km<sup>1/2</sup>, (114.29 m/km<sup>1/2</sup>), Table 1.

Table 1. The basis of the Cuverak basin relief parameters

The name of the basin: Cuverak	
The lowest point of the main watercourse and basin (B), m	385
The highest point of the main watercourse (C), m	754
The highest point of the basin (E), m	793
Average basin main watercourse bed slope ( $I_a$ ), %	13.9
Mean basin altitude ( $M_{sl}$ ), m	646.34
Mean basin altitudinal difference (D), m	261.34
Mean basin slope ( $I_m$ ), %	29.6
Coefficient of basin relief erosion energy ( $E_r$ ), m/km <sup>1/2</sup>	114.29

The following agent of erosion, geologic substrate, with characteristics and diversity contributed to the emergence of the process of erosion in Cuverak basin (Table 2).

The geological substrate of Cuverak basin is diabase (1.62 km<sup>2</sup>, i.e. 100.00%), characterized by properties that is poorly permeable rock, which contributes to the non-resistance soil erosion process. Coefficient of geological substrate water permeability ( $S_1=1.00$ ), indicates nonresistance of Cuverak basin (Table 2).

The soil properties to a lesser or greater extent, contributed to the erosion process. In the area of Cuverak river basin, effects of pedogenetic factors are present as well as skeletoidal brown soil on the diabase. Group of shallow soil of Cuverak basin belongs to brown skeletoidal soil on diabase, with the profile type of Ah-C. In this soil of the studied basin a strong degree process of erosion was represented (Šekularac, 2000).



Table 2. The Cuverak basin geological substrate, coefficient of water permeability ( $S_1$ ) and erosion resistance

The name of the basin: Cuverak		
F <sub>ppr</sub> -Poorly permeable rocks		
• Diabase	km <sup>2</sup>	1.62
	%	100.00
Coefficient of geological substrate water permeability ( $S_1$ )		1.00
Resistance of geological substrate to erosion		Nonresistant

The elements of climate which contribute to the occurrence of the process of soil erosion are rainfall, air temperature and soil temperature. The average annual rainfall (R) in Cuverak basin is 770.2 mm, and the mean annual air temperature (t) is 8.4<sup>0</sup> C, which indicates that these two elements of climate play an important role on soil erosion in study area.

Representation of the another factor of the process erosion, vegetation, both domestic as well as those of anthropogenic origin, and vegetation cover coefficient ( $S_2$ ), are shown in Table 3.

Table 3. The structure of the Cuverak river basin according to the plant cadastre and vegetative cover coefficient ( $S_2$ )

The name of the basin: Cuverak			
F <sub>f</sub>	Forests and coppice of good spacing	km <sup>2</sup>	0.90
		%	55.55
	Orchards	km <sup>2</sup>	0.15
		%	9.26
F <sub>g</sub>	Meadows	km <sup>2</sup>	0.07
		%	4.32
	Pastures and devastated forests and coppices	km <sup>2</sup>	0.35
		%	21.61
Σf <sub>g</sub>		km <sup>2</sup>	0.57
		%	35.19
F <sub>b</sub>	Barren land	km <sup>2</sup>	0.15
		%	9.26
	Infertile soil	km <sup>2</sup>	0.00
		%	0.00
Σf <sub>b</sub>		km <sup>2</sup>	0.15
		%	9.26
Vegetation cover coefficient ( $S_2$ )		0.71	

The total area under forests and coppice of good spacing ( $\Sigma F_f$ ) in the basin Cuverak is 0.90 km<sup>2</sup> (55.55%), the amount of grasses vegetation ( $\Sigma F_g$ ) is 0.57 km<sup>2</sup> (35.19%), and the barren land

( $\Sigma f_b$ ) is 0.15 km<sup>2</sup> (9.26%). That indicates that the studied area is well protected from the effects of erosion processes: coefficient of vegetative cover,  $S_2$  is 0.71 (Table 3).

In which scope the potential of Cuverak watercourse presents great destructive power and a factor of erosion, can be indicated by elements of hydrographic and hydrological characteristics of the study area. Characteristics of family of torrential flow of the Cuverak basin are:  $F_b$ : E; IV;  $Z=0.31$  which means that Cuverak torrential flow is IV class with destructive erosion coefficient ( $Z$ ) of 0.31 (weak intensity of erosion processes, deep type).

Due to all these characteristic of Cuverak basin a certain amount of sediment is produced and certain intensity of erosion is manifested. Size of process erosion of the Cuverak river basin is shown by mean annual erosion sediment ( $W_{year}=646.05$  m<sup>3</sup>/year). Measured midterm the total volume of sediments ( $G_{year}$ ), which reaches the mouth of Cuverak is 277.80 m<sup>3</sup>/year, and the total specific annual erosion sediment that reaches the mouth of the Kamenica ( $G_{yr/sp}$ ), is 171.48 m<sup>3</sup>/km<sup>2</sup>/year. These data indicate that from the Cuverak basin areas disappear per year 0.323 ha of soil by the effect of erosion, the depth of 0.2 m, and 0.06 mm soil disappears from the basin area per year. In addition to acceptance of the mean bulk density of 1.5 g/cm<sup>3</sup> per year 0.48 t/ha is lost.

### Conclusion

The Cuverak torrential flow has its own specific features: IV class destructiveness, coefficient of erosion ( $Z$ ) 0.31, which indicates the strength of weak erosion of deep type. The above, and other factors of erosion of the basin has contributed that annual erosion sediment was 646.05 m<sup>3</sup>/year, while the intensity of erosion was 171.48 m<sup>3</sup>/km<sup>2</sup>/year.

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**AMPELOGRAPHIC AND AMPELOMETRIC CHARACTERISATION OF GRAPES OF  
VITIS VINIFERA L. NATIVE OF ALGERIA**

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**Abstract**

Algeria has many indigenous varieties of vine that their valorization still remains to be undertaken. Homonyms and synonyms have been highlighted in studies of molecular characterization. Our study is part the objective of the ampelographic characterization of 15 cultivars of vines belonging to the collection of the regional station *with* Benchicao (Area of Medea), for identification and determination of the relationships between them and grouped according their similarities. This study is conducted using 112 quantitative descriptors and 14 qualitative descriptors established by the OIV. A statistical study was conducted to highlight the most discriminant parameters, namely, angles, depth of sinuses in relation to the lengths of veins. However, the principal component analysis and hierarchical classification have permits to group the varieties in 4 Groups more or less distinct, which we brought out the presence of clones among the individuals analyzed. This diversity of native varieties put to a severe test, risk extinction if their preservation is not undertaken immediately.

**Keywords:** diversity, *Vitis vinifera* L, varieties indigenous, valorization, characterization ampelographic, Ampélogométric.

**Introduction**

The cultivation of the vine occupies an important place in the traditions of *Maghreb* peasant populations in general and Algeria in particular. Since its extension to Phoenician times, Carthaginian and Roman times, these productions especially wine have become a source of income next to the culture of the olive and fig trees. A wine export market and other crops have been established between the cities of North Africa and Rome. In Algeria, as a result of different mutations undergone by the agricultural area, the diversity of plant genetic resources is in a worrying state of regression or even of disappearance. This situation is to combine the effects of globalization, which has caused a reduction in the diversity of native varieties. In the perspective of identifying the diversity within native varieties and their possible conservation in collection, we took for our characterization study fifteen individuals of *Vitis vinifera* L., using different approaches (Ampelographic and ampelométric).

These minor local varieties are an important resource of genes for viticulture. Their genotypes may have an interest in winemaking in addition to the advantage of their adaptation to local conditions. The variability could be heterogeneous origin. It could be the result of domestication hermaphrodite directs the female individuals of *Vitis vinifera* subsp *sylvestris*, wild subspecies of *Vitis vinifera* L., or the produces of different crosses between domesticated varieties (native flore). But we have no doubt that this wealth could come from the introduction of non-native varieties from antiquity to the 19th century in the Mediterranean countries. These introductions may have been accidentally crossed with local varieties. This hypothesis corroborates with the results of work

characterization performed in Europe on the genetic variability of the specie in the Mediterranean (Grassi et al, 2008, 2003 Crespan et al 2006 ... De Mattia et al 2009, 2007, Zecca et al. 2009).

This varietal component as large and complex remains unknown, unexploited where many varieties are at risk of extinction (Imazio and al.2006).

This development necessary because of often imprecise descriptions related to abundant synonyms, eliminates the existing errors to level historical and Ampelographic

Our characterization answers the problems of risk of loss of genetic diversity in *Vitis vinifera* L. Indigenous varieties subject to progressive varietal erosion due to the ignorance of their agronomic and technological qualities, because this genetic diversity is poorly documented and most of these genotypes have been no studies to assess their importance.

### **Materials and Methods**

The plant material is collected at the regional station of the Technical Institute of Fruits Trees and vine Benchicao (Medea). The 15 cultivars subject of this study are referred to as following: Ahmar Bou Ameer, Amellal, Bezoul El Khadem, Cherchali, Tiziouinine, Benchicao Ahmar, Muscat d'El Adda, Sid Ahmed Draa El Mizan, Ek.Has (Amokrane) Timeskine, Aneb El Kadi, Lakhdari, Ferrana black, Toutrisine and Adari.

The vines (10 repetitions per variety) were randomly arranged and planted in an experimental plot, cultivated in espalier and grafted with the rootstock SO4, with Guyot pruning. This plot is very homogeneous in terms of soil composition and topography.

#### **Ampelographic parameters**

All Ampelographic characters used in our study according to the codification of (OIV 1983) for a description of the adult leaves and budding are 15 in number and are: 067 (AG 06), 070 (AG 07), 071(AG 08), 072 (AG 09), 075 (AG 10), 076 (AG 11), 079 (AG 12), 80 (AG 13), 082 (AG14), 083-1 (AG15), 016 (AG 05), 007(AG 01), 008 (AG 02), 009 (AG 03), 010 (AG 04) .

#### **Ampélogométric parameters**

Sampling of adult leaves was carried out between nouaison and veraison. Parameters Ampelographic were observed and measured on the herbaceous shoot, the mature leaf. We selected 10 sheets per variety. Several studies show that this is a sufficiently representative number of samples (Branas, 1974; Galet, 2000a et 2000b 1983; Dettweiler, 1991; Martínez et Mantilla, 1994). The selected leaves were between 8th and 12th node, counting from the base of the branch. The basis of measurements, according Cid-Alvarez et al. (1994) and Martinez and Grenan (1999), rests on 92 parameters (length of the ribs, angles, serrations, serrations reports) measured by individualizing those corresponding to the left side and those on the right side and that, in order to highlight any variations between the two sides of the sheet, completed by twenty (20) indices indentation on the left side and the right side (Fig .1).

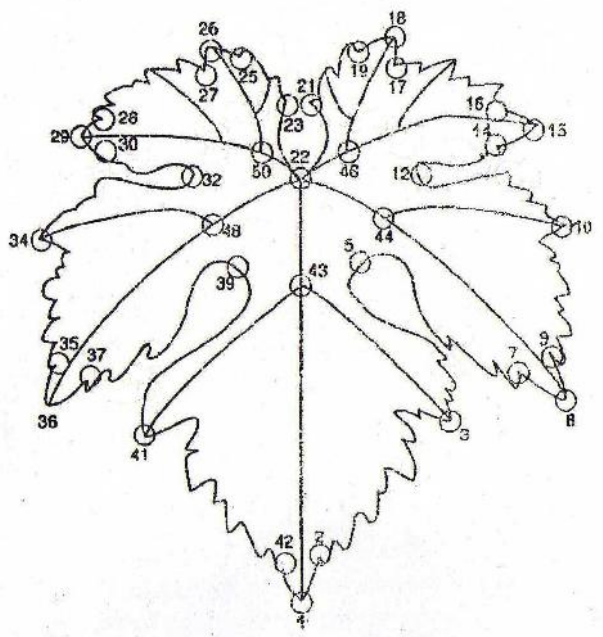


Figure 1. Schema of representative points of the sheet to calculate ampélogométrics parameters (Romani et al 1992).

The leaves are dried, put in herbarium and were photographed with a Panasonic digital camera (Lumix DMC-FZ8) in similar lighting conditions, distance and speed. The different measurements were performed by digital image analysis using the software tool 3 UTHSC. (Rotaru L., 2005).

#### Statistical Analysis

We conducted an analysis of variance, separately for each parameter using the XLSTAT 2012 software and software Stat.Box, 6.40 backed a factor analysis of correspondence (AFC), a principal component analysis (PCA) and a hierarchical classification (C.A.H).

### Results and discussion

#### Ampelographic parameters

Descriptive study of Ampelographic parameters, it seems that the shape of blade (067 OIV parameter) and teeth (076 OIV parameter) and the distribution of anthocyanin ribs (OIV parameters 070.071) give a dispersion more at least importante of varieties studied, which is consistent with the results obtained by Cid-Alvarez et al, 1994 and Santiago et al., 2007.

The factorial analysis of qualitative variables shows that apart from the color of the ventral face of node, leaf shape and the shape of the serrations which have considerable contribution at discrimination more at least clear among the cultivars, the rest of the variables involved little the dispersal of individuals along the axes F1 and F2, but they could still make out the lot individually the following varieties: Ahmar of Benchicao, Sid Ahmed Dra El Mizan, Cherchali and Timeskine (Fig. 2).

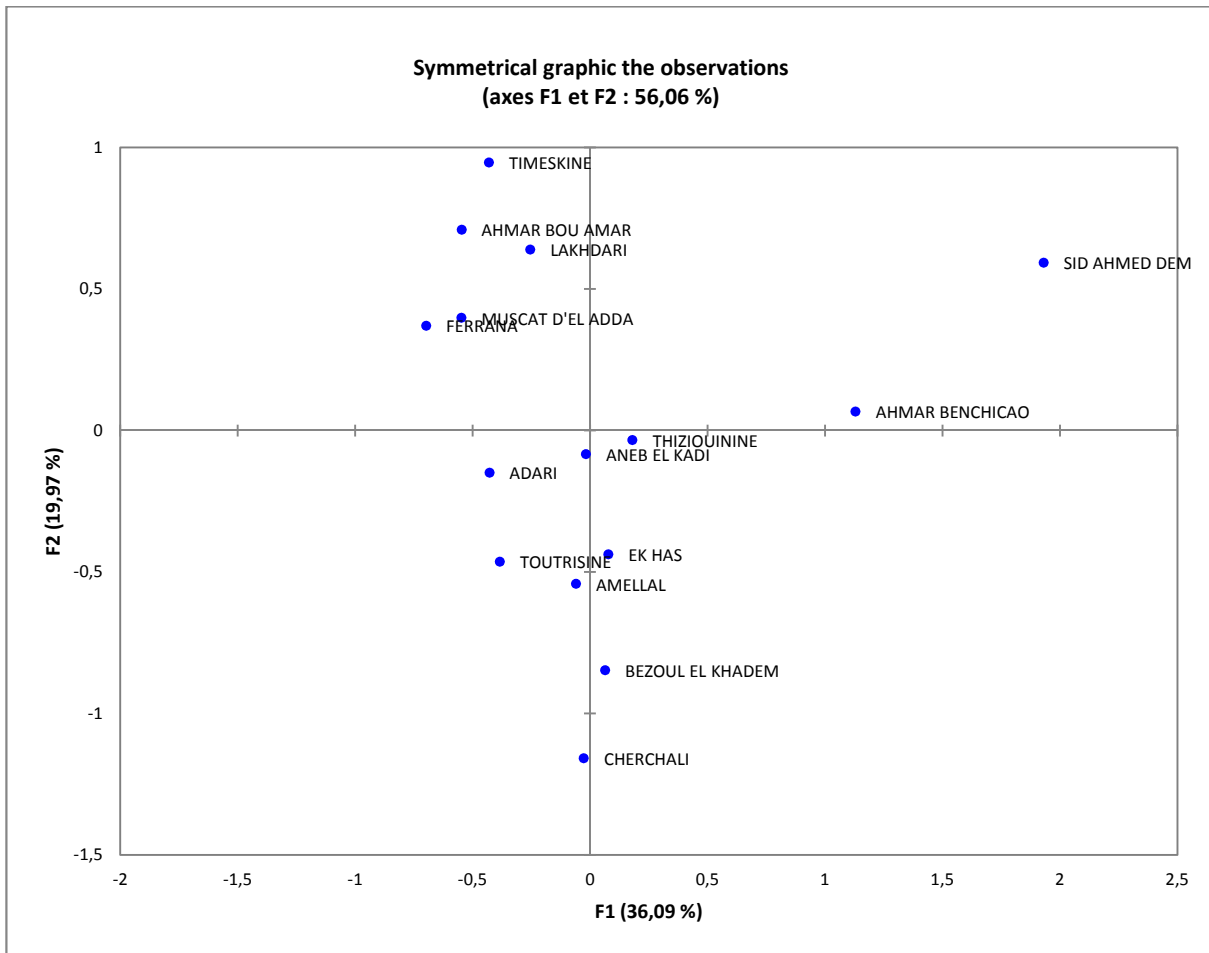


Figure 2. Graphical representation of the varieties studied (AFC)

#### Ampélogométric parameters

The analysis of variance (ANOVA) at the 5% has enabled us to compare the averages in the fifteen varieties studied and showed that there are significant differences for most parameters ampélogométric used in case of our study. Quantitative variables were able to differentiate between individuals studied with discrimination more or less apparent on two axes F1 and F2 that give us 50.04%. Especially varieties Ahmar Bouamar, Ferrana noir, Toutrisine and Adari (Fig.3).

AN (sheet width)  $X3g = (S1g S2g +) / ( + )$  and parameters related to leaf angles are variables which the highest discriminatory power compared to other variables submitted to the study because according to (Martinez et al., 1997 Boursiquot et al.,1989), they are not influenced by climate, pedological nature of the soil and maintenance of soil

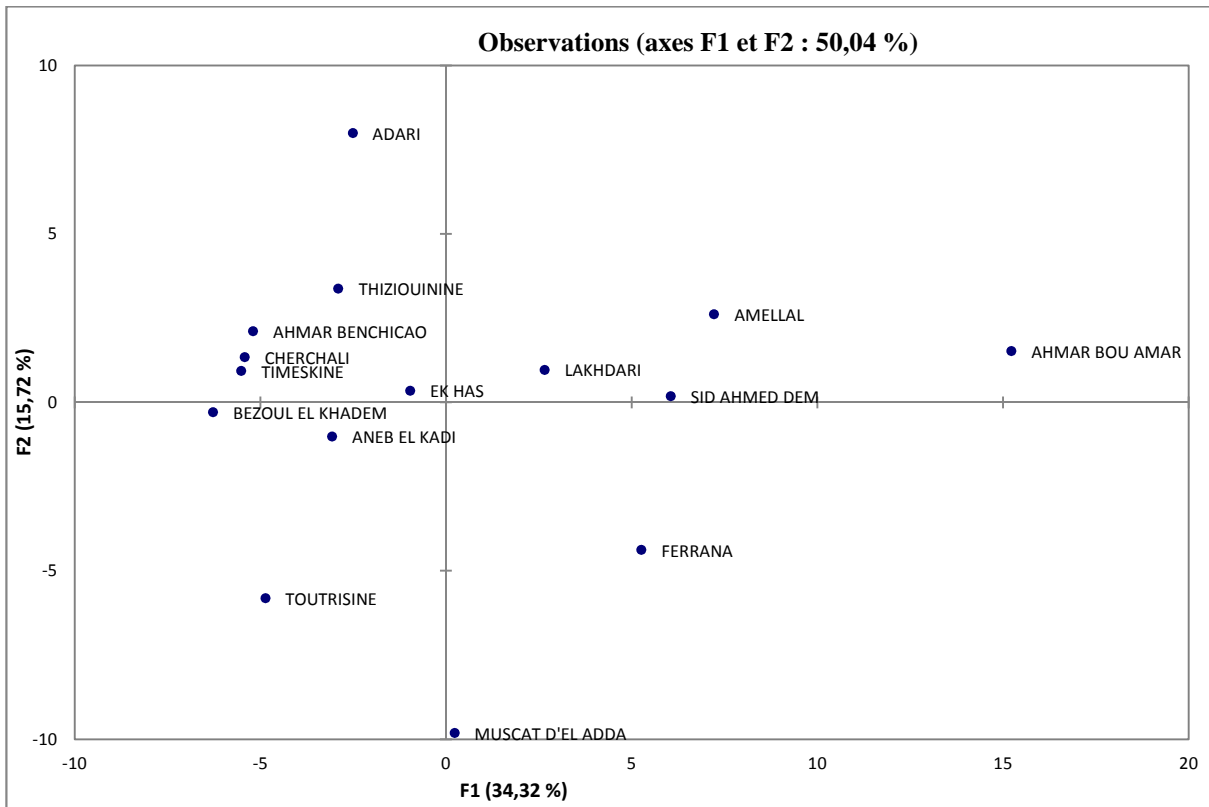


Figure -3: Graphical representation of the varieties studied (PCA).

#### Hierarchical clustering

The elaboration of the dendrogram of hierarchical classification of varieties (Fig.4) was performed with the principle of minimal loss of inertia. from Analysis of the dendrogram, following the method of Rotaru (2009), shows the existence of three optimal groups (classes):

Group 1: represented by varieties Amellal (w), Adari (w), Bezoul El Khadem (B),Tiziouinine, Ahmar of Benchicao (R),Cherchali (B), Timeskine (w), Aneb El Kadi (w), Lakhdari (w) and Ek.has (Amokrane) (w),

Group 2: includes grape variety Ferrana black berries.

Group 3: contains the varieties Toutrisine (w),Ahmar Bou Amar (R),Muscat d'El Adda(w),and Sid Ahmed Draa El Mizan (w).

The three main groups (branches) can be characterized as follows:

Group 1: The grapes Amellal, Adari are very close to each other, by what they have the highest index of similarity (0.998) and the nearest of 1, so they are morphologically similar, because these varieties are white grapes.

Group 2 represented by Ferrana variety that is a black grape berries.

Group 3: the grapes varieties Sid Ahmed Draa El Mizan (w) and Ahmar Bou Amer (R) which are close to one another, but the first one is white and the other is a black grape variety. two different grape varieties by the color of their berries

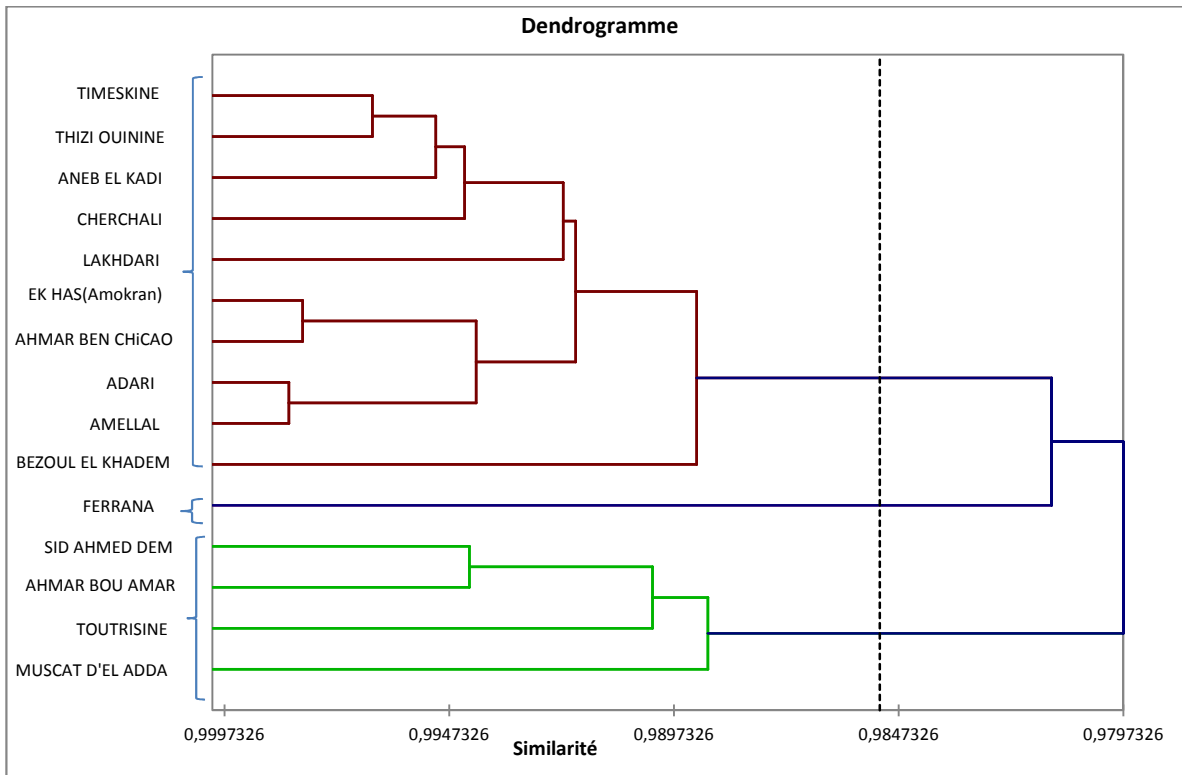


Figure 4: Hierarchical clustering

### Conclusion

In conclusion, our characterization work by ampelographic and ampéométric both approaches are complementary, is a very useful tool for differentiating the varieties studied.

The ampelographic study of various organs of individuals studied showed the existence of a foliar polymorphism and morphological differences among cultivars studied.

Although the classic ampelography is still the most common method used in a practical way by many people especially vines specialists, ampéométric complements these data (Cid-Alvarez et al., 1994).

Through the results of various statistical analyzes of ampéométric parameters studied, it appears that the native varieties are phenotypically different.

Indeed, the analysis of the different parameters studied estimated that the angles and the reports are more effective for the differentiation between varieties.

Similarly, analyzes have highlighted the parameters Ng (Number of secondary teeth on the left side), AN (sheet width)  $X3g = (S1g S2g +) / ( + )$ , among the leaf parameters as a variable that has the highest discriminatory power compared to other variables subject to study.

The approach we have used appreciable if we take into account assessments formulated by Cid-Alvarez et al. (1994). According to him, the use of identification keys varietals from measurements or ampéométric data seems valid for a particular area or region defined with a limited number of varieties, because you should know that the components of the climate have much influence on the force which greatly influences on the parameters studied. Therefore, it is not possible to consider the development of a valid key. This led to the search for new and more accurate methods of characterization (Sefc KM et al., 2001), as the characterization through microsatellite markers that provides a useful advantage when it comes to establishing the kinship between cultivated and wild grape and end the problems of synonymy between varieties.



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## EFFECT OF ALTITUDE ON THE WATER BALANCE OF LAND AREA OF SARAJEVO

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### Abstract

This paper examined the impact of altitude on the water balance of the land area of Sarajevo (weather station Bjelave and weather station Bjelasnica) for the period 1991-2010 for the average, driest and rainiest hydrological year. The average annual potential evapotranspiration (ETP) in the weather station Bjelave was 529 mm, while in the weather station Bjelašnica potential evapotranspiration (ETP) value was much lower, amounting to 54 mm.

In the area of weather station Bjelave, the mean annual precipitation (P) for an average hydrological year amounted to 1.144 mm, potential evapotranspiration (ETP) 595 mm, and actual evapotranspiration (ETR) to 522 mm. Water shortages in the area covered by the weather station appeared in the summer months (July and August), total of water of 158 mm, whereas the excess water in the soil, due to its water saturation occurred in the winter months (December, January, February) and in March and April, its total of 389 mm.

In the area of weather station Bjelašnica, the annual precipitation means (P) for an average hydrological year amounted to 955 mm, potential evapotranspiration (ETP) 70 mm, and actual evapotranspiration (ETR) 68 mm. Shortages of water were not present in a single month, and excess water might occur during all the months except October, in the amount of 719 mm.

**Key words:** altitude, evapotranspiration, water balance, lack of water, excess water

### Introduction

Of all the natural factors, the air has the highest impact on the crop production. For the climate assessment climate of an area, long-term observation and knowledge of its elements, such as mean annual and mean monthly temperatures, amounts, i.e. total precipitations, number of days without frost, the frequency and intensity of the wind, the onset of hail, are needed. Crop production is mostly determined by the air temperature and precipitations, so the climate of a region may be assessed, accordingly.

According to Milosavljevic (1983), the area of Sarajevo is characterised to have pre-mountainous climate, with cold winters, lasting longer than the continental ones. The winds are rare, with moderately warm summers of the annual fluctuations in temperature, being 20-24°C. The average annual temperature is below 10°C, with the annual rainfall of 750-1000 mm, which corresponds to sub-humid climate.

The weather conditions determine the natural flow of water into the soil and water consumption on evapotranspiration and thus irrigation is needed. The most important are precipitations because they need to ensure a steady flow of water for normal growth and development of plants. Different plants have different water needs. The required amount of water for the cultivation of agricultural plants matches the values of potential evapotranspiration.

Agricultural crops requirements for water are expressed through evapotranspiration (Doorenbos and Pruitt, 1977), which includes plant transpiration and evaporation (evaporation) of the land covered with vegetation cover.

The increased quantity of water used in the ETP is usually accompanied by increase in the yield of agricultural plants to a certain level, when there comes to a stagnation. Then, the yield decreases, despite increased ETP values.

Based on the numerous analyses, for most sites it seemed sufficient to have values of minimum and maximum daily air temperatures (Popova et al., 2006; Jabloun and Sahli, 2008; Trajkovic et al., 2011). Measurements of air temperature are simple and are not prone to big errors, unlike other climate parameters.

The most commonly used methods are those of Thornthwait, the Turc, the Blaney-a - a-Criddle and Penman. In this paper, the calculation of potential evapotranspiration by the Thornthwaite method was used, giving the best results in terms of sub-humid climate.

Thornthwaite's method is applied worldwide, being very suitable because only the data on the mean daily air temperature calculated as the average value of the maximum and minimum air temperature, are necessary. Camargo et al. (1999) reported that the method of Thornthwaite, on a monthly basis, seemed more reliable, if instead of daily mean air temperature, the effective temperature was taken into account.

In this paper, an initial assumption was that climatic factors were very important in agricultural production, and that determining the type of climate and water balance calculation seemed to be very important in order to obtain information on shortages and excess soil water in a particular area, with the resulting need of introducing either watering or, if there need be, land drainage.

### **Materials and method**

The data on climate elements (air temperature and precipitation) of the area of Sarajevo (Bjelave and Bjelasnica) on a twenty-year basis (1991-2010), were used. Bjelave weather station is placed at an altitude of 630 m, whereas that of Bjelasnica is located at an altitude of 2067 metres. Based on the collected climatic data for each weather station, the potential evapotranspiration for each month and each year analysed, was determined. Also, based on the ETP value and rainfalls, water balance was determined as well as the water shortages and surpluses of the area.

The potential evapotranspiration was determined using Thornthwaite's method, based on the air temperatures values, so that the incorrect ETP was corrected with a certain coefficient for a given area.

Water balance was determined by the method of Thornthwaite, on the basis of two parameters: the monthly value of ETP (mm) and the sum of monthly precipitation (mm) for the analysed period.

Water balance was made for the reserve of readily available water (RRLW) in the soil of 100 mm, and the land was then assumed to be saturated with water.

### **Results and discussion**

In farming, significant results are achieved thanks to the increasing advances in the field, but still the output is not stable due to water shortages during the growing season, because water is necessary for normal plant growth and development. The lack of moisture for plants is due to the high temperatures, low relative humidity, high values of potential evapotranspiration and insufficient rainfalls.

Precipitation is a meteorological element, the value of which is highly variable over space and time. The influence of topography is very important, rainfall increases with altitude, because the hills affect the air currents rise, leading to air cooling and condensation of water vapor. The nature and dynamics of rainfall also affects the temperature regimes.

The average annual ETP in Bjelave was found to be 529 mm, while in the Bjelašnica ETP value was much lower of 54 mm. The annual heat index values were also relatively low, because of low mean monthly temperature in Bjelašnica, and that is why the ETP value was very low compared to the value of the ETP of the weather station Bjelava.

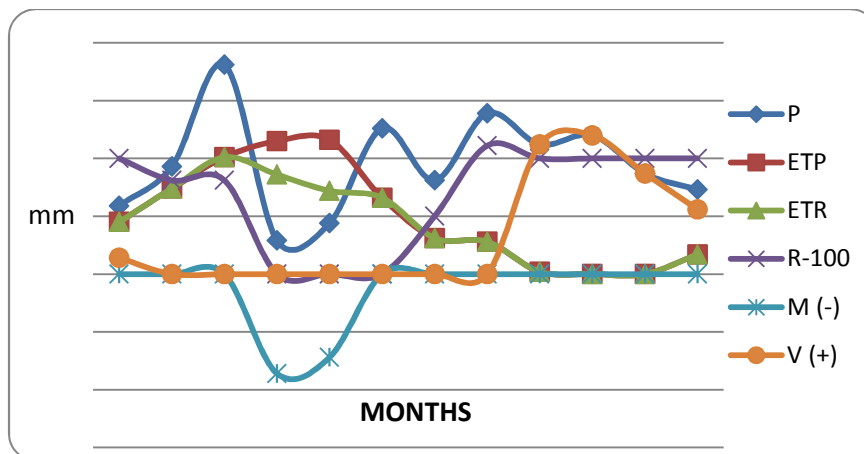


Figure 1. Water balance of the weather station Bjelava for the average hydrological year (2010)  
 P- precipitation, ETP-potential evapotranspiration, ETR-actual evapotranspiration, R-100- reserwe water in the soils, M (-)- water shortages, V(+)- surplus of water

In the area of weather station Bjelavea total annual amount of precipitation (P) was 1.144 mm, potential evapotranspiration 595 mm, and actual evapotranspiration (ETR) 522 mm. Water shortages in the area covered by the weather station appeared in the summer months (July and August), with the total sum of 158 mm, while the surplus of water occurred in the winter months (December, January, February) as well as in March and April, with their total amount being 389 mm, since the earth was then completely saturated with water (Fig. 1).

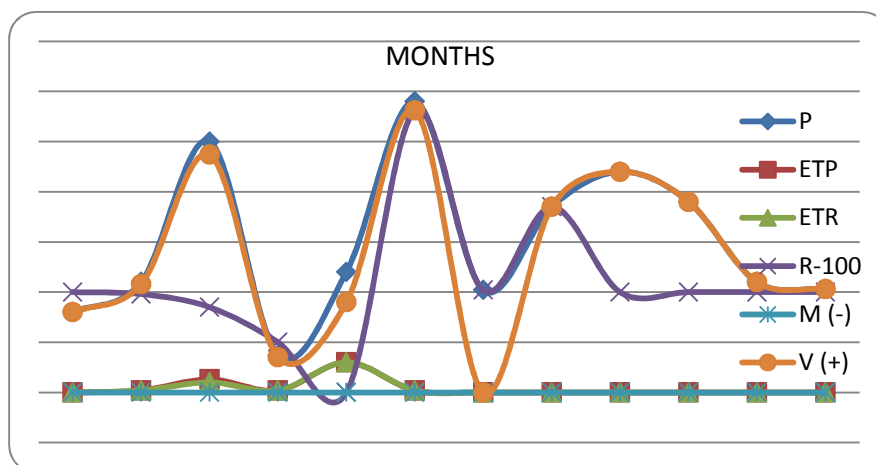


Figure 2. Water balance of the weather station Bjelašnica for the average hydrological year (2010)

In the area covered by the weather station Bjelašnica, the annual amount of rainfall was much higher amounting to 1.797 mm. Since a lot of low air temperature prevailed in this area because of higher altitudes, the average annual potential evapotranspiration (ETP) was only 49 mm, and the

actual annual one (ETR) 46 mm. Shortages of water were not present in a single month, and excess water could occur in all months except for October, amounting to 1,639 mm per year.

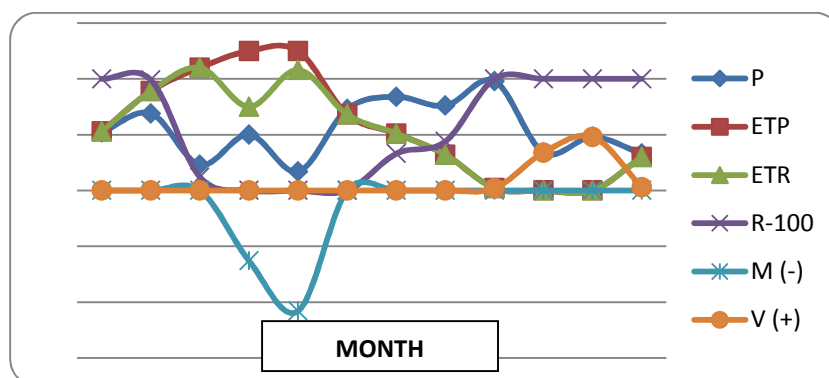


Figure 3. Water balance of the weather station Bjelava for the driest year (2000)

On the area of the weather station of Bjelava, the total annual precipitation sum (P) for the driest year amounted to 657 mm, potential evapotranspiration was 657 mm, and the effective one (ETR) 618 mm. Water shortages in the area covered by the weather station appeared in the summer months (July and August), with the total sum of 171 mm, whereas the surplus of water occurred in the winter months (December, January, February, March) in the amount of 87 mm, (Fig. 3)

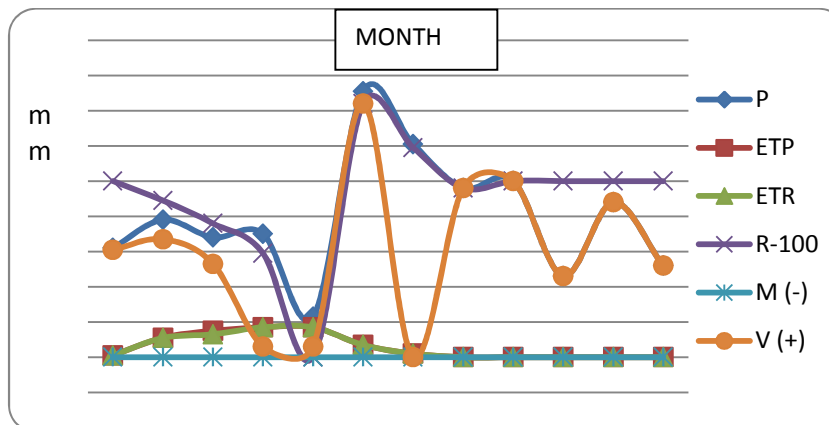


Figure 4. Water balance of the weather station Bjelašnica for the driest year (2000)

In the area covered by the weather station Bjelašnica, the mean annual precipitation (P) was 955 mm, potential evapotranspiration (ETP) 70 mm, and actual evapotranspiration (ETR) 68 mm. No water shortages were recorded in a single month, whereas its surpluses were in all months except October, but in much less quantity than being in the rainy years, with the surplus water per year being 719 mm (Fig.4).

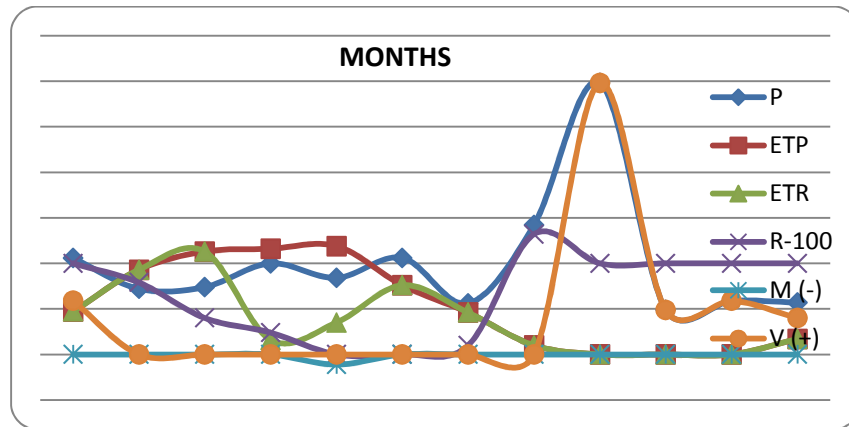


Figure 5. Water balance of meteorological station Bjelave for the rainiest year (1999)

In the area Bjelava, total rainfall was 1,203 mm during the year, potential evapotranspiration (ETP) 637 mm and actual evapotranspiration (ETR) 453 mm. Water shortages were reported only in one summer month (August), while its surpluses were recorded in the winter months, the total of 505 mm (Fig. 5).

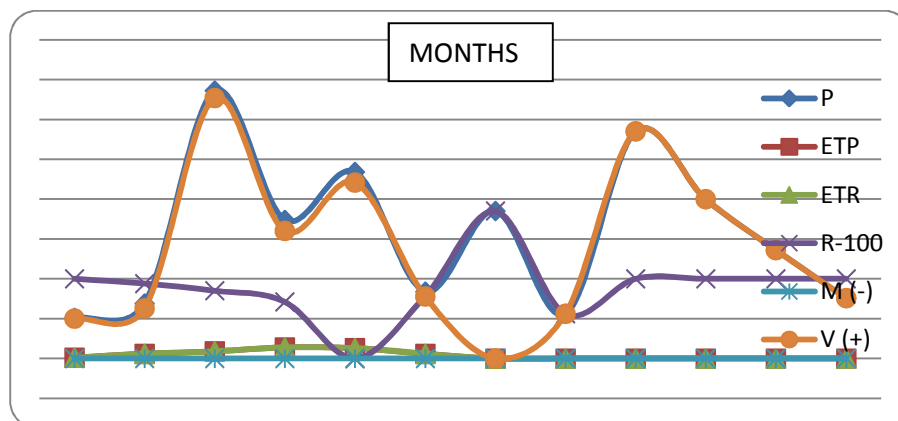


Figure 6. Water balance of the weather station Bjelašnica for the driest year (1999)

In the area of MS Bjelašnica, the annual rainfall was reported to be 1,886 mm, potential evapotranspiration (ETP) 49 mm, and the actual one (ETR) 49 mm. Water shortages were present not in a single month during the hydrological year, but its surplus was in all the months except for October, with the total amount being 1.652 mm (Fig. 6).

### Conclusion

In the area of the meteorological station Bjelave (630 meters), high temperature over the summer caused extremely high values of the potential evapotranspiration, reaching their maximum in July and in August, after which, the decrease in the average monthly air temperatures brought about the decreased potential evapotranspiration value. High air temperatures and high values of potential evapotranspiration led to water shortages in the summer months. Low temperature, the decreased value of potential evapotranspiration and increased precipitation also led to water excess in the soil.

In the area covered by the weather station of Bjelašnica (2067 m), the average annual temperature was significantly lower, and air temperatures were extremely low, too, along with reduced values of the potential evapotranspiration throughout all the months, and increased precipitation, resulting in the water surplus in the soil in almost all the months except for October.

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## EFFECT OF DIFFERENT LAND USE ON MAIN SOIL PROPERTIES

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### Abstract

Intensive agriculture throughout a long period is likely to cause soil degradation. Therefore, monitoring of soil quality in agricultural regions is essential for food quality and food safety. The present study is conducted in the agricultural region of Croatia (Osijek-Baranja County) where 74 samples were collected from forest land (21) and agricultural land (53). The study investigates differences between forest soils and agricultural soils (arable land and pasture) in regard of main soil properties such as pH, soil organic carbon (SOC), bulk density (BD), total nitrogen (Tot-N), available phosphorous (AL-P) and available potassium (AL-K). In addition to main soil properties, total concentrations of several micronutrients (Fe, Mn and Zn) have been observed as well. The aim of this study is to identify potential soil degradation of particular soil property. The significant differences ( $p < 0.001$ ) have been observed for all above mention soil properties (with exception of micronutrients). However, negative effect of agricultural practice has been observed only for SOC, Tot-N and BD, suggesting degradation of these soil properties. As mentioned earlier, total concentrations of micronutrients (Fe, Mn and Zn) showed no significant difference between land use, therefore there is no soil degradation due to agricultural practice with regard of these three micronutrients. However, availability of these micronutrients is largely dependent on soil properties (pH and SOC).

**Keywords:** agriculture, forestry, soil quality, soil properties

### Introduction

Growing world population and increasing demand for food resulted with intensive agriculture practice that, as consequence, had an effect on soil quality and caused soil degradation. When addressing soil quality, it is essential to take into account that soil quality is related to particular soil function (Karlen et al. 2003), so to accurately assess soil quality first we need to know which soil function we are evaluating. For example, if we are evaluating different management practices or land uses we need to focus on soil properties directly affected by land use change (Andrews et al. 2002a; Karlen et al. 2003). Usually, the assessment of soil quality requires as many biological, physical and chemical soil properties as possible (Brejda et al. 2000a, b; Andrews et al. 2002a, b; Shukla et al. 2006; Imaz et al. 2010). In present study we had no information on biological data and physical data was represented only by BD, all other parameters were chemical properties of soil. Therefore, we focused our study on chemical properties of soil that might be influenced by land use change.

The aim of present study is to investigate differences of soil properties between forest soil and agriculture soil and to determine the influence of land use change on soil properties. The study is conducted in the eastern part of Croatia (Osijek-Baranja County), which has been agricultural region for centuries. It is our hypothesis that there will be differences between land uses.



## Materials and methods

### 2.1. Study area

Study area (Osijek-Baranja County) is located in the eastern Croatia. It is a part of the Pannonian Valley that stretches through Hungary, Serbia and Croatia. The county consists of 9 soil types, i.e. Stagnosols, Dystric Cambisols, Luvisols, Haplic Gleysols, Chernozems, Fluvisols, Eutric Cambisols, Mollic Gleysols and Anthrosols (FAO 2006). Based on the pedological map 74 soil samples were taken from all soil types. However, since some of them cover small and insignificant area, the number of samples varied among soil types. The sampling included agricultural sites (53) and forest sites (21). Forest soils were mainly 80-year old oak forest with very little human activity, therefore forest was considered as natural site without human influence (i.e., fertilizers, pesticides, cultivation).

### 2.2. Soil sampling and analysis

The sampling sites were randomly chosen so that all soil types and different land uses of the sampling area were included (arable land and forest). From each sampling site, 10 subsamples from 0–25 cm depth (hereafter called surface soil) were taken within 5 m distance from each other and then combined into one sample of approximately 500 g. Samples were dried and sieved through the 2-mm sieve for the determination of soil pH, AL-P and AL-K. For determination of soil organic carbon (SOC), total nitrogen (Tot-N) and total metal concentration in soil ( $M_{tot}$ ), samples were further ground to finer particle size using agate mortar. The concentration of SOC was determined by dry combustion method on a LECO Carbon Determinator EC12 (Nelson and Sommers 1982), Tot-N concentrations were determined by LECO CHN-1000 Carbon and Nitrogen Analyzer. Soil pH was determined in soil to water solution ratio of 1:2.5 (Mc Lean 1982). Available P and K were determined by ammonium lactate (AL) extraction method (Egner et al., 1960.). Bulk density (BD) was determined by core method. Analysis of soil samples for pH, SOC, Tot-N, and trace metal concentrations was conducted at the Norwegian University of Life Sciences, while the determination of BD, AL-P and K at the University of J.J. Strossmayer, Faculty of Agriculture, Osijek, Croatia.

Soil samples for total metal concentration of Fe, Mn and Zn ( $M_{tot}$ ) were digested in concentrated ultra pure  $HNO_3$  (1:15 solid:solution ratio) by stepwise heating up to 250°C using a Milestone Ultra CLAVE for 1 hr and 15 min. The concentrations of trace metals were then determined by using a Perkin Elmer Optima 5300 DV Inductively Coupled Plasma Optic Emission Spectrometer (ICP-OES). Standard reference material (SRM) used was the SRM 2709 (National Institute of Standards & Technology 2003).

### 2.3. Statistical analysis

Descriptive statistics, analysis of variance (ANOVA) and Tukey pairwise comparison of means was conducted using Minitab<sup>®</sup> Statistical Software version 15 (Minitab 2007). Analysis of variance and Tukey pairwise comparison was done between land uses to determine soil parameters with significant differences. In addition, GIS technique was used to create maps of the area and to visualize the results. The maps were created in ArcGis version 9.2. (ArcGis 9.2. 2006), a software that combines table data with spatial data, allowing us more comprehensive insight into a particular area of interest (Hutchinson and David 2000).

## Results and discussion

The results show that all of the investigated soil properties (with exception of trace metals) differ between forest and agricultural soils. Forest soils have better BD, SOC and Tot-N while agricultural soils have higher values of available P and K, as well as pH (Table 1.). Better pH in agricultural soils is a result of liming practice which helps in keeping the pH at desirable levels for agricultural production, while better AL-P and AL-K is due to the P and K fertilization. Forest higher SOC is mainly due to constant input of organic materials such as leaves and fallen branches (forest litter). Decrease in SOC, which is organic matter, in agricultural fields can affect nutrient cycling, pesticide and water retention as well as soil structure (Karlen et al. 1997). Information on BD is showing that agricultural soils are more compact, which means they are degraded compared to forest soils since BD can effect root penetration, water- and air-filled space and biological activity.

Table 1. Descriptive statistics of soil properties for different land use ( n = 74)

	Land use	n	Mean	StDev	Min	Max
<b>pH</b>	Agri. field	53	6.8	1.003	4.3	8.02
	Forest	21	5.2	0.825	4.4	7.40
	<b>ALL</b>	<b>74</b>	<b>6.3***</b>	<b>1.180</b>	<b>4.3</b>	<b>8.02</b>
<b>SOC</b> (%)	Agri. field	53	1.5	0.7	0.46	4.4
	Forest	21	2.3	0.8	0.94	5.1
	<b>ALL</b>	<b>74</b>	<b>1.7***</b>	<b>0.8</b>	<b>0.46</b>	<b>5.1</b>
<b>Nitrogen</b> (%)	Agri. field	53	0.15	0.07	0.04	0.44
	Forest	21	0.23	0.10	0.10	0.56
	<b>ALL</b>	<b>74</b>	<b>0.17***</b>	<b>0.09</b>	<b>0.04</b>	<b>0.56</b>
<b>AL-P</b> mg/100kg	Agri. field	53	25.5	29.8	0.00	174.2
	Forest	21	5.9	6.5	0.00	29.0
	<b>ALL</b>	<b>74</b>	<b>19.91***</b>	<b>26.91</b>	<b>0.00</b>	<b>174.2</b>
<b>AL-K</b> mg/100kg	Agri. field	53	23.8	12.8	8.5	84.6
	Forest	21	13.5	5.4	7.6	28.7
	<b>ALL</b>	<b>74</b>	<b>20.9***</b>	<b>11.6</b>	<b>7.6</b>	<b>84.6</b>
<b>BD</b>	Agri. field	53	1.46	0.13	1.14	1.71
	Forest	21	1.27	0.15	0.91	1.45
	<b>ALL</b>	<b>74</b>	<b>1.42***</b>	<b>0.16</b>	<b>0.91</b>	<b>1.71</b>
<b>Fe</b> mgkg <sup>-1</sup>	Agri. field	53	29736	3423	23909	40221
	Forest	21	27769	5624	19642	41034
	<b>ALL</b>	<b>74</b>	<b>29178<sup>ns</sup></b>	<b>4220</b>	<b>19642</b>	<b>41034</b>
<b>Mn</b> mgkg <sup>-1</sup>	Agri. field	53	639	200.6	237.8	1144
	Forest	21	713	299.5	218.5	1459
	<b>ALL</b>	<b>74</b>	<b>660<sup>ns</sup></b>	<b>233.2</b>	<b>218.5</b>	<b>1459</b>
<b>Zn</b> mgkg <sup>-1</sup>	Agri. field	53	80.88	14.06	58.29	119.5
	Forest	21	78.57	22.34	52.29	122.4
	<b>ALL</b>	<b>74</b>	<b>80.23<sup>ns</sup></b>	<b>16.69</b>	<b>52.29</b>	<b>122.4</b>

\*\*\* indicate significant difference between land uses at  $p < 0.001$  respectively while *ns* stands for “not significant”

As mentioned earlier the significant differences ( $p < 0.001$ ) have been observed for all investigated soil properties with exception of micronutrients. However, total concentration of micronutrients does not represent the available fraction. Bioavailability of these micronutrients is

largely dependent on soil properties such as pH and SOC (Ivezic et al. 2012; Sauvé et al. 2000). Therefore, influence of liming on pH and negative effect of agricultural practice on SOC can have effect on availability of micronutrients (Fe, Mn and Zn). Thus, we can argue that availability of micronutrients is also affected by land use change (Ivezic et al. 2011).

To make the issue of soil quality more understandable to non-experts and policymakers, mapping of soil quality can provide simple visual insight in the current situation of soil. Mapping is a useful approach in describing spatial and temporal dynamics of soil properties (Cambardella and Karlen 1999; Wanyama et al. 2005). Considering above mentioned results, GIS technique of mapping soil properties is a reliable tool for spatially presenting the results (Figure 1.) and potential changes in land use through time.

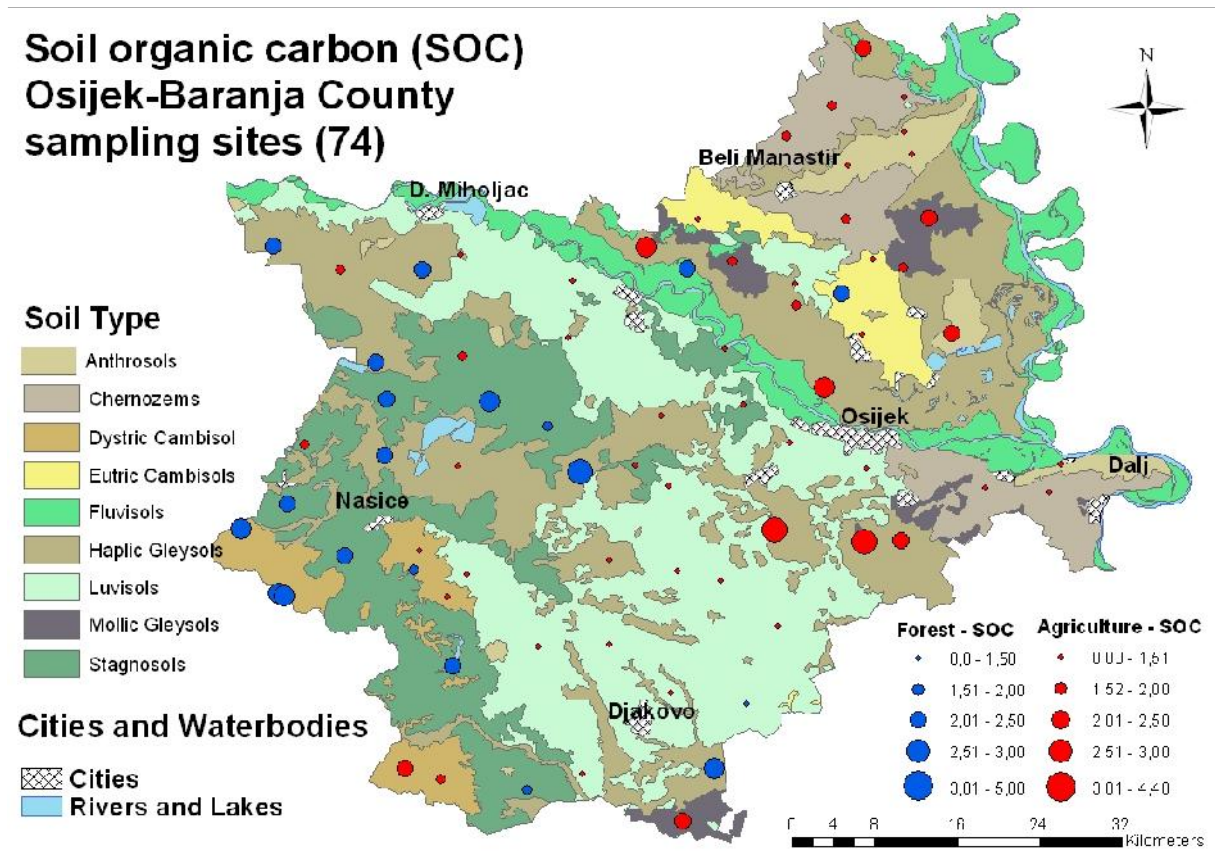


Figure 1. Soil organic carbon concentrations in Osijek-Baranja County

The dependence of SOC on land use can be observed from the maps (Figure 1). However, statistical analysis is necessary for confirmation of significant differences seen in the map. In a same way other soil properties can be presented as well.

### Conclusion

In present study, investigated soil properties showed significant differences between land use (forest and agriculture). Negative results of some soil properties (SOC and BD) in agricultural soils indicated soil degradation due to agricultural practice. However, it is our opinion that the situation is not alarming and that there is no overall degradation of soil quality as agricultural soils showed better results for some other soil properties (AL-P and AL-K, as well as for pH which was adjusted for agricultural practice). In conclusion, we can say that the soils of main agricultural region of Croatia have not been seriously degraded due to long period of agricultural activity.

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## HUMUS COMPOSITION OF CHERNOZEM, EUTRIC CAMBISOL AND LUVISOL IN CONTINENTAL CROATIA

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### Abstract

The aim of this study was to determine humus composition in the surface layer of Chernozem, Eutric Cambisol and Luvisol, major soil types used for crop production in continental Croatia. The study was conducted on 36 soil samples collected at 12 locations, of which 4 in each soil type. Humus content was determined by modified Walkey-Black method (1934) and the humus composition was analyzed by the version of Schnitzer method (1982) using a mixture of 0.1M NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>. UV-VIS spectroscopic characterization of humic acid and fulvic acids was performed at wavelengths 465 and 665 nm. Average humus content decreased in the following order: Chernozem (2.99%) > Eutric Cambisols (2.23%) > Luvisols (1.71%). The average carbon content in humic acids decreased from 32.7% (Chernozem) to 14.72% (Luvisols), while in fulvic acids ranged from 15.97% (Chernozem) to 24.91% (Luvisols). The average ratio of humic acid and fulvic acids (Ch/Cf) decreased from 2.05 established in Chernozem to 0.60 in Luvisols. Structures of higher aromaticity – humic acids prevail in Chernozem and Eutric Cambisols, whereas aliphatic structures characteristic of fulvic acids are prevalent in Luvisols. The average ratio of optical densities (E<sub>4</sub>:E<sub>6</sub>) ranged from 2.4 (Chernozem) to 6.02 (Luvisols). Data obtained by the non-invasive - spectroscopic method are in agreement with data of carbon content in humic acid and fulvic acids obtained using the modified Schnitzer method.

**Key words:** humic acid, fulvic acid, Ch/Cf, E<sub>4</sub>:E<sub>6</sub>

### Introduction

Humus composition defines key soil characteristics and its fertility, and is an indicator of the processes in soil. Soils differs in the amount and composition of humus, and this diversity is closely related to human activity such as soil tillage, hydro ameliorations, fertilization, crop rotation etc. Monitoring of humus composition in soil is the key to understanding and predicting the future state of the soil. The aim of this study was to determine humus content and composition in the surface horizons of Chernozem, Eutric Cambisol and Luvisol, major soil types used for crop production in continental Croatia.

### Material and methods

The study was conducted at 12 locations, on which were taken 36 soil samples, four for each soil type (Chernozem, Eutric Cambisol and Luvisol). A three average soil samples were taken from depth 5-20 cm at each location. Soil samples were prepared for laboratory analysis according to HRN ISO 11464:2009. Humus content was determined by modified Walkey-Black method (1934) and the humus composition was analyzed by modified Schnitzer method (1982) using a mixture of 0.1M NaOH and Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>. UV-VIS spectroscopic characterization of humic acid and fulvic acids was performed on spectrophotometer SHIMADZU UV-1700, at wavelengths 465 and 665 nm. The total nitrogen content was determined by Kjeldahl method (HRN ISO 11261:2004). The soil samples were also analyzed on basic physical and chemical properties. Soil texture was determined by sieving and sedimentation (HRN ISO 11277:2004), the pH in distilled water and 1M solution of KCl (HRN ISO 10390:2005), and the base saturation of adsorption complex by Kappen method (JDPZ, 1966).

## Results and discussion

Basic chemical properties of soils and their texture are shown in Table 1. Chernozem has silty loam texture and weakly acid to neutral pH. The humus content is low and medium, average low (2.99%). Texture of Eutric Cambisol is silty loam, pH is acid to neutral, base saturation of adsorption complex is high and humus content low. Luvisol has also silty loam texture, very acid to acid reaction, low to medium base saturation of adsorption complex and very low humus content.

Table 1: Chemical properties and soil texture of P1 horizons

Soil type	Statistics	pH		V* %	Humus %	Percentage of mechanical particles diameter				
		H <sub>2</sub> O	KCl			2,0- 0,2	0,2- 0,063	0,063- 0,02	0,02- 0,002	<0,002
Chernozem	Mean	7,29	6,33	-	2.99	0.7	1.5	36.2	31.7	25.8
	Min	6,76	5,54	-	2.40	0,3	0,6	34,0	28,5	23,3
	Max	8,07	7,13	-	4.00	1,1	2,6	38,7	34,3	27,9
Eutric Cambisol	Mean	6,46	5,31	79,4	2.23	7.8	12.4	33.1	32.2	28.1
	Min	5,51	4,32	65,1	1.30	1,1	1,5	20,3	29,8	14,1
	Max	7,54	6,70	93,3	2.80	10,8	17,7	40,8	34,9	33,7
Luvisols	Mean	5,46	4,33	37,9	1.71	10.2	14.1	38.7	30.3	17.4
	Min	4,41	3,65	16,1	1.00	0,4	1,7	30,4	27,5	6,5
	Max	6,57	5,48	55,6	2.20	16,6	19,0	46,7	35,6	22,5

\*base saturation of adsorption complex

Properties of selected soils are very similar to typical (average) soils of wider research area described by Škori et al (1977).

Low humus content in P1 horizon of Chernozem and Eutric Cambisol, as well as very low humus content in Luvisol are results of human activity such as deep ploughing, fertilization and crop rotations with reduced proportion of grasses, as pointed by Kibblewhite et al (2005). These data fit into status and trends of humus in anthropogenized soils of Europe. Around 45% of the mineral soils in Europe have low or very low organic carbon content (0-2%) and 45% have a medium content (2-6%) (Rusco et al 2001). 74% of the land in southern Europe is covered by soils that have less than 2% of organic carbon in the topsoil (Zdruli et al 2007).

Statistical data for total inorganic carbon and nitrogen, C/N and humus composition in P1 horizon of studied soils are shown in Table 2.

The C/N ratio in the studied soils are in agreement with earlier research of Škori et al (1977) who determined range of C/N ratio 10-13 in Chernozem and 9.5-15 in Eutric Cambisol of eastern Slavonia. Slightly wider C/N ratio in relation to the above mentioned study was determined only in Luvisol (average C/N 13.7) while mentioned authors listed C/N range 10.1-11.2.

Table 2: Mean minimal and maximal values of total inorganic C, nitrogen, C/N, humic acid, fulvic acid, Ch/Cf, humin and E4/E6 in P1 horizons

Soil type	Statistics	C org %	N %	C/N	Ch %	Cf %	Ch/Cf	Humin %	E4/E6
Chernozem	Mean	1.74	0.167	10.4	32.69	15.97	2.05	51.34	2.47
	Min	1.39	0.142	8.4	27.90	13.20	1.59	45.00	2.23
	Max	2.32	0.221	12.8	37.00	18.50	2.41	58.20	2.92
Eutric Cambisol	Mean	1.29	0.116	11.2	24.69	20.83	1.20	54.48	3.81
	Min	0.75	0.066	9.4	21.90	18.00	1.02	50.60	3.37
	Max	1.61	0.166	13.5	27.40	24.10	1.52	60.10	4.18
Luvisol	Mean	0.98	0.073	13.7	14.72	24.91	0.60	60.38	5.98
	Min	0.57	0.041	11.1	10.80	21.30	0.43	55.90	5.17
	Max	1.26	0.114	16.8	17.40	28.90	0.73	65.00	6.43

The average proportion of humic acid, fulvic acid and humin in humus of Chernozem, Eutric Cambisol and Luvisol is shown on Figure 1-3.

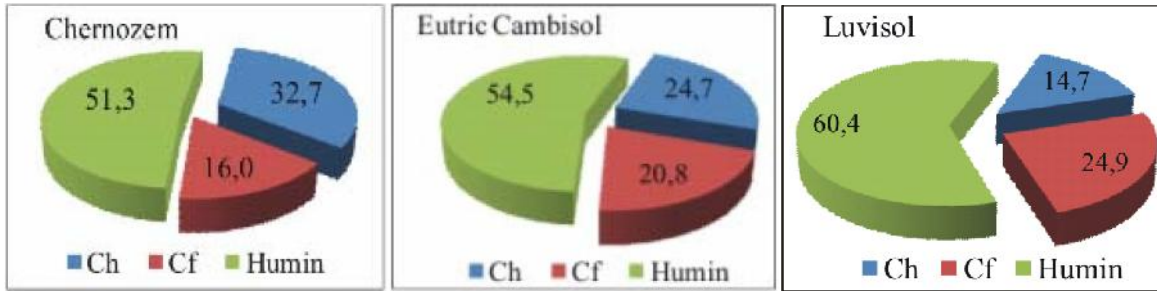


Figure 1-3: The average proportion of humic acid, fulvic acid and humin in humus of Chernozem, Eutric Cambisol and Luvisol

The average carbon content in humic acid decreased from 32.69% in Chernozem, over 24.69% in Eutric Cambisol to 14.72% in Luvisol, while in fulvic acid increased from 15.97% in Chernozem to 24.91% in Luvisol. The dominance of humic acid over fulvic acid in humus of Chernozem was recored in research of Pospišilova and Fasurova (2009), Nikolaishvili and Matchavariani (2008) and Gonet et all (2008).

The average ratio of humic acid and fulvic acid (Ch/Cf) decreased from Chernozem (2.05) over Eutric Cambisol (1.20) to Luvisol (0.60). About a similar ratio of humic acid and fulvic acid in humus of mentioned soils reports Stevenson (1994).

The relationships between humus fractions – humic acid, fulvic acid and humin are one of the most important indicators of humus properties in different soil types. Visualization of their relationships was given in the triangular diagram such as one for soil texture by Watanabe and Kuwatsuka (2001). In the 3D diagram (Figure 4) plots of humus composition of Chernozem, Eutric Cambisol and Luvisol were clearly separated. The diagram show that the separations between soil types are results of differences in all humus fractions, but the most pronounced are in humic acids. Humus composition presented in 3D diagram provides a clear visualization of the relationships between studied soil types.

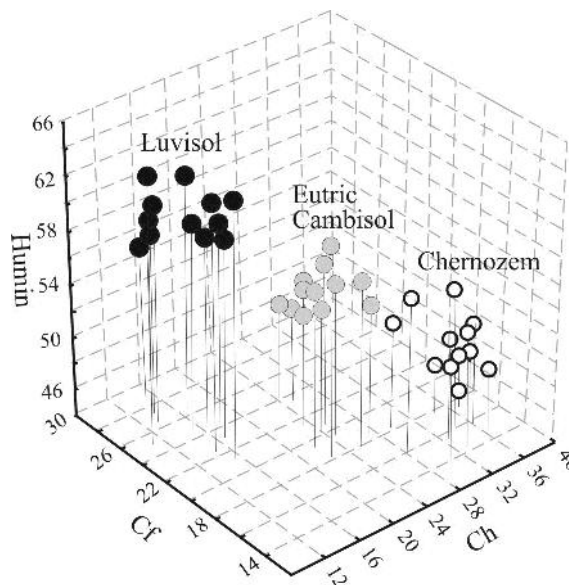


Figure 4: 3D diagram for humus composition of Chernozem, Eutric Cambisol and Luvisol



Quantitative values of humic acid and fulvic acids, as well as their relationships were compared to UV-VIS spectroscopy method. Figure 5 shows  $E_4/E_6$  ratio defined as the ratio of optical absorbance at 465 nm to that of 665 nm for humic substances in aqueous solution. This ratio has been correlated negatively with increasing content of condensed aromatic structures (Bravard and Righi 1991; Stevenson 1994). Therefore, the more refractory and complex the soil humus, the lower should be its  $E_4/E_6$  ratio. Described index was the lowest in Chernozem and the highest in Luvisol. Higher degree of aromaticity in humic acid of Chernozem, in comparison to Eutric Cambisol and Luvisol in their study showed Baran ikova et al (1997).

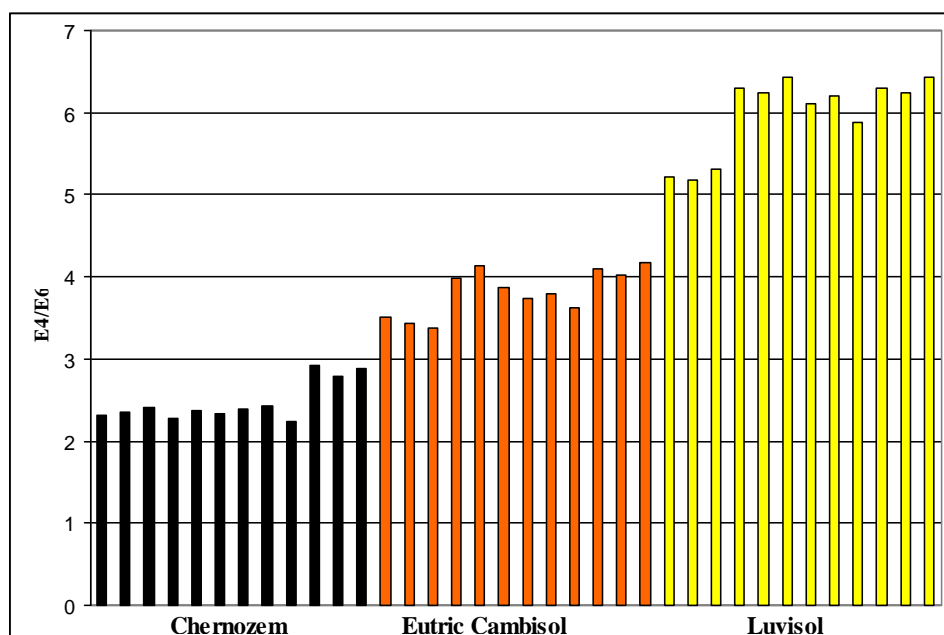


Figure 5: The optical indexes ( $E_4/E_6$ ) calculated as the ratio of  $A_{465} / A_{665}$  nm in soil samples

### Conclusion

The average humus content in studied soils are low and very low and decreased in the following order: Chernozem (2.99%) > Eutric Cambisol (2.23%) > Luvisol (1.71%). In humus of Chernozem and Eutric Cambisol prevail humic acid (Ch/Cf 2.05 and 1.21), while in Luvisol Ch/Cf is 0.60. UV-VIS spectroscopically established ratio  $E_4/E_6$  confirms above mentioned relationships of humus fractions. Humus composition refers to clear differences between the studied soil types and plot of humus fractions presented in 3D diagram provides a clear visualization of their separation.

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## LAND SUITABILITY FOR OLIVE IRRIGATION. CASE STUDY: POSTIRA ON THE ISLAND OF BRAČ

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### Abstract

The most relevant method to improve olive production in Mediterranean climate conditions is irrigation. The aim of this study was to evaluate the land suitability for olive irrigation in an area of 482 ha located in the northern part of island of Brač (Postira municipality). Olive trees have been sparsely planted without irrigation with densities of traditional groves between 70 and 150 tree/ha, and the soil periodically tilled. Evaluation of land suitability was based on detailed soil surveys and analyses of natural characteristics (terrain, climate, geology, hydrology and land use) according to FAO concept. In the research area dominant soil type is Terra rossa formed on the Cretaceous limestone and dolomites, partly terraced on steep slopes. The factors influencing the land suitability for irrigation were: physical soil properties (texture, soil depth/rooting depth), stoniness, rockiness that determine permeability and available water capacity, slope and possibility of using mechanized tillage. Integration, processing and analysis of soil and terrain parameters were performed in the GIS environment. This research has shown a wide range of land suitability for olive irrigation. Land suitable for olive irrigation covers area of 139.0 hectares (28.5% of total area). Moderately suitable, temporarily and permanently not suitable land covers 105.3 ha or 21.8% of total area. The largest area (237.7 ha or 49.3%) covers permanently not suitable land for olive irrigation. Conducted qualitative assessment of land suitability for olive irrigation is a good basis for decision-making and individual farmers in planning the development of olive production.

**Key words:** land, suitability, irrigation, olives

### Introduction

Soil water deficit, especially in the summer –during the vegetation period, is one of the most limiting factors of agricultural production in the Mediterranean climate. In the Croatian Mediterranean region olive is the dominant crop, usually in extensive farming on marginal lands. Municipality of Postira has the biggest olive production on the island of Brač. It is a typical example of the described conditions and the irrigation is necessary agro-technical measure for its improvement. The aim of this study was to evaluate the land suitability for olive irrigation.

### Study location

Postira municipality is located in the northern part of island of Brač (Adriatic Sea, Croatia) and occupies 480 ha. Investigated location has a Mediterranean climate (Csa) in the Köppen climate classification with hot, moderately dry summers and mild, wet winters. The average annual rainfall was 734 mm (Split, 1981-2000). Indicator of extreme drought in summer period is the lowest monthly average rainfall in July (22 mm). The average annual temperature for the study period was 16.2° C, with a monthly mean temperature of the warmest month - July 26.1° C and 2698 hours of annual sunshine.

Geologically, the investigated area is made of Cretaceous - Senonian limestone and crystalline dolomites and Quaternary Colluvium skeletal deposits (Marin ic, et all, 1969). Geomorphological structure is characterized by typical karst phenomena and can be distinguished following units: a very steep slope, terraced slopes, flat plateaus and narrow and elongated uvale and doči. The largest area occupied terrains with steep slopes (15-25 degrees). Although, there is no surface water streams, because very developed and expressed relief forms represented a large number of torrents.

## **Material and methods**

### Data sources

The evaluation was conducted using the following: Soil map at the scale 1:5,000 (Miloš, B. 2006), Digital ortho-photo (DOF), 1:5000; Basic Topographic Map of Croatia 1:5000 (BTMC); Geological map 1:100,000 (Marin ic, et all, 1969). Data on the structure of agricultural production and the land use of the municipality Postira were taken from the Census of Agriculture (2003) and the Spatial Plan of the municipality Postira in 2002.

### Field work

Field pedocartographic research was carried out according to the methodology of making detailed soil maps. A total of 32 soil samples were taken from 18 soil profiles during the soil survey.

### Laboratory investigation

On collected soil samples laboratory analysis were carried out by following methods: pH (ISO 10390:2004), humus content (Kotzman method), total carbonate content (ISO 10693:2004), content of available phosphorus and potassium (ammonium lactic method, JDPZ, 1966) and texture (ISO 11277:2004).

### Integration, processing and analysis in the GIS environment

GIS is typically used to store and analyze spatially referenced data in a map based format. The entire cycle can accordingly be subdivided in four stages: 1. Collecting geo-spatial data; 2. Transfer the collected data to a format that is appropriate for further processing (preprocessing); 3. Analysis of the land parameters, and 4. Visualisation, editing and presentation. Based on the digital terrain model (DTM) developed in GIS environment using the topographic map (BTMC) at 1:5000 we have created maps of slope and elevation. Map of olive groves we have made by digital ortho-photo (DOF). In this research overlay method has been used to evaluate land suitability. Overall the database is ready to use by the software package Arc View 3.x. All spatial data are geo-referenced to the national coordinate system (Zone 6 Gauss-Krueger projection).

### Land evaluation

Land suitability for olive irrigation is based on the FAO's framework for land evaluation (FAO, 1976) and subsequent guidelines (FAO, 1983 and FAO, 1985). This is considered to be a standard reference system in land evaluation throughout the world (Purnell, M.F. 1979; Dent and Young, 1981; van Diepen et al., 1991; Smyth, A.J. and Dumanski, J. 1993). Land suitability for irrigation of olive groves was performed using soil parameters (soil depth and rooting depth, soil texture, rockiness and stoniness), topographic (slope and elevation), growth conditions (age and vitality of olive groves, the possibility of additional planting) and the possibilities of applying of mechanized tillage.

## **Results and discussion**

### The characteristics of the olive orchards

Spatial distribution of olive orchards is shown on enclosed map (Figure 1). The total area of olive orchards is 482 ha with 52.346 olive trees, average 109 trees per ha. The fundamental feature of the

olive orchard is large spatial variability of all olive indicators: age, vitality, vigor, appearance, growth technology, spacing between trees etc., on small area and especially their large spatial fragmentation. The lush and vital olive trees are in suitable soil conditions, on steep slopes with shallow soils dominated old and scrubby trees with poorly developed crown. The growth and yields of these olives are low and irregular.

#### The characteristics and classification of the studied soils

Variety of soil forming factors, especially of topography, parent material and human impact resulted in large variability of soil (and land) properties important for olive groves (soil and rooting depth, chemical and physical properties, rockiness and stoniness of terrain). Based on field soil survey (Miloš, B. 2006), laboratory analysis of soil samples and using soil classification (Škoric, A. et al 1985) soils of described location we classified as follows: Anthropogenic soil from Terra rossa on limestone and dolomites, Anthropogenic soil terraced from Terra rossa on limestone and crystalline dolomite and Anthropogenic soil on Quaternary colluvium. Subdivision into lower units is according to criteria relevant for their fertility: soil depth and gravel.

#### Anthropogenic soil from Terra rossa on limestone and dolomites

The basic morphological feature of this soils is characteristic reddish brown color (2.5 YR3/4 and 5YR 3/4; Munsell Soil Color Chart). Soil depth varied from extremely shallow (<15 cm) to deep (>70 cm) and it is related to layering of limestone and dolomites, as well as slope. Analyzed soils are non carbonate to medium carbonate with weakly acid to alkaline reaction. The entire soil profile has a very good humus supply while physiologically active nutrients phosphorus and potassium varied in a very wide range (Table 1). These soils dominantly have clayey and silty clayey texture and very good expressed polyhedric structure. Soils varied from non gravel to extreme gravel (>75% of gravel). Important limiting factors for mechanized soil tillage, rockiness and stoniness, varied in wide range 0-75%.

#### Anthropogenic soil terraced from Terra rossa on limestone and crystalline dolomite

The essential feature of these limestone-dolomites sediments is lower water permeability in relation to typical limestone. That is why they keeps more water and provides lateral percolation. Surface P1 horizons of these soils are reddish and reddish brown color (2,5 YR3/4 and 5YR 3/4) typical for previously described Terra rossa. These soils have a low content of carbonates and neutral to weakly alkaline reaction. They also have a good supply of humus like Terra rossa on limestone, medium stokes of potassium and low content of physiologically active phosphorus (Table 1). According to soil texture analyzed soil samples are non gravel and weakly gravelly loams to clay, in average loamy clay with polyhedric structure.

#### Anthropogenic soil on Quaternary colluviums

These soils occupied doce and uvale at the base of steep slopes. The properties of these soils are defined by composition of colluvial sediments, in particular by relationship between limestone gravels and soil particles.

These soils are dominantly extremely deep and gravelly. Total content of carbonate and soil reaction varies depending on limestone gravel content. Analyzed soils have low to high carbonate content, in average medium (15.4% in P1 horizon and 16.2% u P2 horizon) and neutral to alkaline reaction. Humus content is medium. Physiologically active potassium and phosphorus in these soils also varies greatly and in some samples have a high values which indicates strong and irrational fertilization, in particular by phosphorus. Gravel content varies from low (<25%) to extreme (>75%). According to soil texture, these soils are gravelly and very gravelly clayey loams and gravelly clays.

Table 1: Mean minimal and maximal values of chemical properties and soil texture of anthropogenic soils

Soil type Horizon/ depth	Statistics	pH		CaCO <sub>3</sub>	Humus	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	Soil texture			
		H <sub>2</sub> O	nKCl					Coarse sand	Fine sand	Silt	Clay
				%	%	mg/100g.	2-0.2	0.2-0.02	0.02-0.002	<0.002	
Antr. soil from Terra rossa P1(0-30)	Mean	7,82	6,82	2,2	6,83	33,9	11,8	15,1	20,1	22,7	42,1
	Min	6,53	5,36	0,4	3,43	18,0	0,3	3,1	13,1	13,7	27,9
	Max	8,39	7,28	9,6	10,67	60,2	48,8	30,2	27,4	30,5	61,8
P2(30-60)	Mean	8,07	7,00	5,2	6,37	20,5	6,3	11,4	19,7	25,9	43,1
	Min	7,55	5,80	0,4	4,43	11,3	1,0	2,3	7,1	13,3	21,7
	Max	8,33	7,72	27,0	8,70	46,1	22,6	33,9	30,1	40,0	65,5
Teraced soil from Terra rossa P1(0-30)	Mean	8,05	7,15	11,4	6,44	28,7	1,8	16,2	24,1	25,3	34,4
	Min	7,85	6,65	0,4	4,16	21,2	0,3	3,1	18,5	19,7	20,2
	Max	8,39	7,38	22,9	8,35	46,6	2,9	26,8	33,3	30,5	51,5
P2(30-60)	Mean	8,24	7,31	8,1	6,78	14,9	1,45	18,4	19,9	26,5	35,3
	Min	8,15	7,10	1,7	5,68	11,3	1,0	3,7	19,1	24,5	21,7
	Max	8,33	7,72	25,4	7,88	18,5	1,9	33,1	20,7	28,5	48,8
Antr. soil on the Quat. colluvium P1(0-30)	Mean	7,92	7,19	15,4	7,42	89,4	68,0	17,5	17,6	24,9	42,0
	Min	7,83	7,12	8,3	4,60	63,5	57,1	8,4	12,1	7,8	33,2
	Max	8,02	7,29	35,1	8,77	121,9	80,1	30,4	24,7	35,6	49,7
P2(30-60)	Mean	8,08	7,29	16,2	5,61	60,0	15,5	14,5	15,6	27,6	42,2
	Min	7,91	7,12	7,8	4,10	27,0	2,2	6,1	9,3	13,0	29,1
	Max	8,25	7,44	36,3	7,47	121,9	32,8	41,5	19,3	36,7	64,6

### Topography

Topographic analysis included the calculation and making maps of the slopes and elevation. The area and percentage share of each class of inclination and elevation are shown in Tables 2-3.

Table 2: Area of the olive orchards inclination classes

Inclination (degree)	<2	2-6	6-9	9-12	12-17	17-24	24-33	>33	Total
Area (ha)	67	82	75	61	60	72	60	3	482,0
Percentage (%)	14,0	17,1	15,6	12,7	12,5	15,0	12,5	0,6	100,0

Flat terrain and those with gentle slopes (< 9°) occupy 224 ha or 46.5% of area, while steep slopes (>9°) occupy 256 ha or 53.5% of area.

Olives are grown in range from sea level to over 350 m altitude. The water reserves on the land are on altitude of 150 m. Consequently, terrains above that altitude are less suitable for irrigation.

Table 3: Area of the olive orchards elevation classes

Elevation (m)	0-50	50-100	100-150	150-200	200-250	250-300	>300	Total
Area (ha)	7,9	74,8	123,4	185,3	44,0	39,1	7,5	482,0
Percentage (%)	1,6	15,5	25,6	38,4	9,1	8,1	1,6	100,0

### Land suitability for olive irrigation

Evaluation of land suitability for olive irrigation is based on expert review and GIS tools. Summary of the land evaluation for olive irrigation appears in Table 4-5 and in Figure 1. Limiting factors are numerous and serious, such as soil depth, slope, rockiness and stoniness.

Table 4: Land suitability classes and limitations for olive irrigation

Class suitability	Description of the limitation*
S1	Highly suitable. Without limitations
S2	Moderately suitable. Moderately serious limitations (d/rd, g)
S3	Marginally suitable. The limitations are serious (d/rd, g, sr)
N1	Not currently suitable. Land with limitations (d/rd, s, g, sr, etc) that could be eliminated by technical means or investment, but that these changes are at present unfeasible
N2	Permanently not suitable. Serious limitations of generally a physical nature, which are assumed to be beyond solving over the long term (d/rd, s, g, sr, etc, e)

\*d- soil depth; rd- rooting depth; g- gravel; s – slope; sr- stoniness and rockiness, e- elevation: etc- olive tree condition

Four mapping units of suitability were separated, of which three were complex (made up of two or more suitability classes). Land suitable for olive irrigation (Suitability order S; mapping units S1-S2 and S2-S3) covers area of 139.0 hectares (28,5%). Complex unit (Moderately suitable, temporarily and permanently not suitable land (S3-N1/N2) covers 105.3 ha or 21,8% of total area. Permanently not suitable land for olive irrigation occupy 237.7 ha or 49.3% of total area. These results show that the area of land suitable for olive irrigation is very limited.

Table 5: Description and area of mapping units of land suitability for olive irrigation

Class suitability	Mapping units of land suitability for olive irrigation				Total area
	S1-S2	S2-S3	S3-N1/N2	N2	
Area (ha)	68,3	70,7	105,3	237,7	482,0
%	14.2	14.7	21,8	49.3	100,0

### Conclusion

Conducted evaluation found that the suitable land for olive irrigation on analyzed location are very limited amounted 139 ha or 28.5% of total area. On the rest of area land limitations are such that they cannot be changed (soil depth, slope, rockiness and stoniness). To improve efficiency of management and olive production we suggest implementation of irrigation system on suitable land.

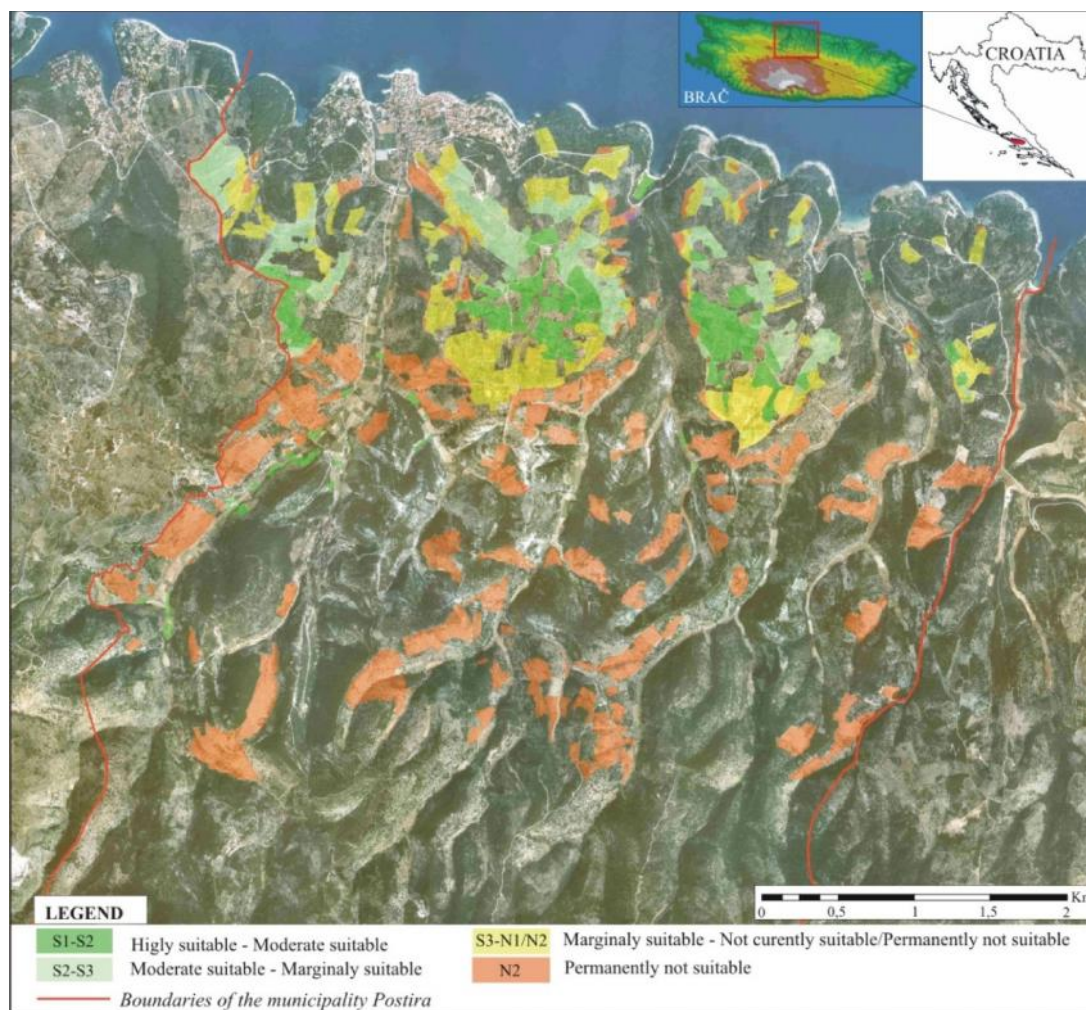


Figure 1: Land suitability map for olive irrigation

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## ASSESSMENT OF THE SOIL SUITABILITY OF ZELINA VINEYARD AREA FOR GROVING GRAPES

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### Abstract

Zelina vineyard area belongs to, according to winegrowing regionalization, north-western part of the Croatian winegrowing, sub region Prigorje-Bilogora, and is important wine growing region in Croatia. Even though there are various possibilities of using land for the purpose of this study attention is focused only on assessment the suitability of the land in Zelina vineyard area for the cultivation for grape growing. Evaluation of soil suitability for Zelina vineyard area was carried out according to the modified FAO method (Brinkman R., Smith, A.I., 1973, Vida ek, Ž., 1976).

In the area of Zelina vineyard 14 pedosystematic units from the division of automorphic and hydromorphic soils were determined. Zelina vineyard area has a total of 31,9 ha of soil suitable for viticulture (class P-1), soil with moderately suitability (class P-2) occupy 3180,8 ha of surface, soil with restricted suitability (class P-3) occupy 1287,0 ha of surface, while permanently unsuitable soils (class N-2) occupy 508,5 hectares of surface. There are no temporarily unsuitable soils (class N-2).

According to the degree of suitability and possibility of usage the most suitable soils for viticulture are eutric brown soil on marl and rendzina on marl and soft limestone with southern, south-western and south-eastern exposure, and on altitude of 150-400 m. The dominant constraints for other units are related to frost, exposure and inclination of the terrain and the some properties of soil. The study shows the hydro and agro melioration measures necessary to implement for individual units of soils to improve their use value.

**Keywords:** land evaluation, Zelina vineyard area, land reclamation

### Introduction

The Republic of Croatia has about 32,485 ha of vineyards (Statistical Year Book, 2012), which represents approximately 2.4% of the used agricultural land. Due to the variety of environmental conditions for growing grapes Croatia is divided regionalization of vineyard areas in the two regions - continental Croatia and littoral Croatia. Zelina vineyard is one of the continental Croatian viticulture region, subregion Prigorje-Bilogora, and it is known for producing quality wines, as well as the festival exhibition of continental Croatian wines – St. Ivan Zelina, which is one of the largest and most important in Croatia.

Grapevine is a longtime culture which for its growth and development needs to the specific requirements in environmental growing conditions - primarily climate and topography. Soil as a third important factor in viticulture, also significantly affects on the quality and quantity of grape production, but it may be different emergency technological measures to adjust the cultivation of grapes. The evaluation and assessment of land for dedicated purposes we can distinguish two types of constraints, namely: limits in space or external characteristics of land (climate, relief) and limitations in the soil or inland properties of solum. Therefore, the main objective of this study was to determine the types and characteristics of soils Zelina vineyards, and evaluate their produced characteristics for viticulture, under certain conditions, relief and climate.

## Materials and methods

For this study it have been used pedological and other existing data which are the property of Soil Science Department and the Central Agricultural Library of Faculty of Agriculture, University of Zagreb. Analysis of climatic features of Zelina vineyard according to the National Weather Service, the weather stations Zelina and Križevci, and includes precipitation, temperature, number of days with frost and insolation for period from 1981 to - 2010th. Systematics of soil was determined according to the current classification of soils (Skoric et al., 1986), and the distribution of soil according Basic soil map of Croatian scale 1:50.000 sections Zagreb 2 and Ptuj 4. Physical and chemical properties of soils are described according to data from previous field and laboratory studies, which are attached to the expositors of soil map. Evaluation of soil suitability classes in the Zelinski vineyard area, taking into account the type and intensity of limitations to their intensive use for viticulture, is in accordance with the modified criteria for soil evaluation (FAO, 1976; Vida ek Ž., 1976).

## Results and discussion

### Geographical position

Zelina vineyard is situated on the north-eastern part of Zagreb County and bordered by the hills of Medvednica, Kalnik and Papuk and the rivers of Sava and Drava and covers hilly landscape of Bilogora.

### Climatic features

Climate is usually the determining factor of growing grapes in vineyard production. Generally it can be said that the climate of Zelina vineyards is a temperate continental, and according to value of Lang rain factor (81.9) has a humid characteristics. The average annual rainfall (period 1981-2010) is 909.6 mm, with a large annual variation of 594.6 mm to 1290.7 mm. During the active growing season grapevine (IV-IX month) average fall 516.4 mm, while the outside of the growing season (X-III month) 393.2 mm. Since the minimum rainfall for growing vines is 300-400 mm, and the optimal amounts is 650-850 mm (Licul R., Premužić D., 1974), precipitation is not a limiting factor for growing grapes in this area. The average annual air temperature (period 1981-2010) is 11.1°C, with the annual variation of 9.7°C to 12.3°C. According to thermal markings it is a moderately warm climate. The average air temperature during the active growing season is 17.7°C, the coldest month is January with an average temperature of -0.3°C, while the monthly mean temperature of the warmest month - July is 21.5°C. Mean daily temperature above 10°C (effective active temperature) occur in April and cease during October. The grapevine is long day plant, and the required about 1500 to 2500 hours of insolation for successful cultivation. Since the average annual value of insolation in the area of Zelina vineyards is 2006.3 hours, here vinegrape is situated in the optimal conditions for development. The highest average insolation in July (295.4), and lowest in December (49.0). In the area of Zelina vineyards there are great variations in the number of frost days, so that in some years during the vegetation period are not recorded frosts, until years when during the vegetation was recorded up to 32 days of frost.

### Relief

The relief of a region characterized by the shape and position of the earth's surface into space. Zelina vineyard is surrounded by the north side position of mountain chain Zagreba ka gora and Kalnik, which lowered to the south are more or less and make the combination of hills and hillock at whose slopes are located vineyards. The positions of vineyards Zelina descend quite uniformly to the south, southeast and east. At these almost the proper deployed ribs eastern part of Zagreba ka gora on mild and sometimes steeper slopes are vineyards on the eastern, southern and western exposures, and inclination of the slopes is quite mild. Therefore this is a vineyard in terms of orographic very suitable for growing grapes. A characteristic of these slopes and hills that always

reduces altitude above sea level going from north to south and from west to east, and the vineyards are located at an altitude of 150 m to 400 m (Melik Z. et al., 2008).

#### Soil

According to the Basic soil map of Croatia scale 1:50 000, section Zagreb 2 and Ptuj 4 in the area of Zelina vineyards found 15 soil mapping units from the division of automorphic and hydromorphic soils, whose spatial distribution is shown on soil map scale of 1:100 000, figure 1. It was found 10 types of soil from automorphic division (rhegosol, colluvial soil, calcomelanosol, rendzina, vertisol, distric cambisol, eutric cambisol, calcocambisol, luvisol and rigosol) and four types of soils from hydromorphic division (pseudogley, pseudogley-gley, humofluvisol and eugley). Basic physical and chemical properties of certain types of soil are described according to the results of previous field and laboratory studies, and are found in expositors of Basic soil map sections Zagreb 2 and Ptuj 4.

**Figure 1:** Soil Map of Zelina vineyards area, scale 1:100.000 with a legend

#### Evaluation of agricultural land for vineyards

##### Concept and evaluation criteria

Land as an object of evaluation includes physical space - climate, relief, soil, geology, hydrosphere, vegetation, and the results of past and present human activities (drainage and irrigation, terracing, deep tillage, fertilization etc.), to the extent of their impact on benefits and features dedicated using (modified according to FAO, 1976, Vidanek, Ž., 1976).

Evaluation of land suitability Zelina vineyards for viticulture is primarily qualitative and based on relevant soil properties and qualities and/or limitations of systematic soil units. Classification structure consists of orders, classes and subclasses suitabilities for viticulture. Orders determine the suitability (P) or unsuitability (N) of soil for viticulture; classes determine suitability degree: P-1 good or suitable soil for viticulture without major restrictions, P-2 moderately suitable soil for viticulture with individual limitations and P-3 limited suitable soil for viticulture with a number of serious limitations.

Class N-1 temporarily unsuitable soils for viticulture that require radically arranging and class N-2 permanently unsuitable soils for viticulture because their arrangement is not possible or not economically justified. Subclasses of soil suitability and unsuitability determine the types and intensity of limitations, which are shown in table 1

**Table 1:** Types of restrictions with the intensity and the criteria used in the evaluation of land suitability for vineyards

<b>Landforms (r)</b> r1 = narrow stream valleys r2 = narrow river valleys r3 = closed depression r4 = plains r5 = hills r6 = mountain	<b>Terrain inclination (n)</b> n1 = 0-1% flat n2 = 1-3% almost flat n3 = 3-8% gentle slopes n4 = 8-16 moderate slopes n5 = 16-30% moderately steep slopes n6 > 30% steep slopes
<b>Ekological depth of soil (du)</b> du1 = very shallow 0-15 cm du2 = shallow 15-30 cm du3 = medium deep 30-60 cm du4 = deep 60-120 cm du5 = very deep >120 cm  <b>Climate (k)</b> - frost, fog	<b>Drinage (dr)</b> dr1 = very weak dr2 = weak dr3 = incomplete dr4 = moderately good dr5 = good dr6 = slightly excessively dr7 = excessively
<b>Soil acidity (a)</b> a1 = very acid < 4,5 a2 = acid 4,6-5,5 a3 = weakly acid 5,6-6,5 a4 = neutral 6,6-7,2 a5 = basic > 7,2	<b>Humus content (hu), %</b> hu1 = very low < 1% hu2 = low 1-3 % hu3 = moderate 3-5 % hu4 = high 5-10% hu5 = very high > 10%
<b>Actively lime (vp), %</b> vp1 = little < 5 vp2 = moderately 5-15 vp3 = much >15	<b>Soil water regime</b> mv = periodic water deficiency in the soil vv = periodic excess water in the soil v = stagnant surface waters pv = flood waters V = high level of underground water
<b>Supply of physiologically active phosphorus, mg P<sub>2</sub>O<sub>5</sub>/100 g of soil (fv)</b> fv1 = poor < 12 fv2 = moderately 13-20 fv3 = good 21-30 fv4 = very good >30	<b>Supply of physiologically active potassium, mg K<sub>2</sub>O/100 g of soil (kv)</b> kv1 = poor < 20 kv2 = moderately 21-35 kv3 = good 36-50 kv4 = very good >50

Results of the assessment and present land suitability

Table 2 shows the results of evaluation of the suitability of agricultural land Zelina vineyards for growing vines respecting the data of the soil properties, relief and climate. Based on identified limitations were determined mapping units of soils of different suitability for viticulture production (Figure 2): P-1 class (mapping unit 11) is very good suitability - area of 31,9 ha, P-2 class (mapping units 4, 6, 16, 21, 22) moderate suitability - area of 3180,8 ha, P-3 classes (mapping units 5, 15, 19, 27) limited suitability - area of 1287,0 ha and N-2 class (mapping units 2, 8, 32, 35, 40) permanently unsuitable soils - area 508,5 ha.

**Table 2.** Suitability of agricultural land of Zelina vineyards for viticulture

Number	SOIL MAPPING UNITS (name and structure)	AREA ha	SUITABILITY CLASSES	THE DOMINANT LIMITATIONS
11	Eutric cambisol on marl Rendzina on marl and soft limestones	31,9	P-1	fv1, kv1, n4
4	Rendzina calcareous and vitisols on marl Rhegosol siliceous-calcareous Vertisol calcareous and noncalcareous, rigosols on marl	1422,2	P-2	n5, du3, vp2-3
6	Rendzina calcareous on marl Anthropogenic rendzina and vitisols Rhegosol siliceous-calcareous Luvisol typical and pseudogleyic on loess	1011,1		n5, du3, vp2, hu2
16	Distric cambisol, luvisol and pseudogleyic, on noncalcareous loess Luvisol typical and pseudogleyic on loams and sand Pseudogley on slope	213,1		k, fv1, kv1, n4
21	Luvisol on loess Rendzina on marl Rhegosol siliceous-calcareous, partly antropogenic soils	387,2		k, fv1, kv1, n4
22	Luvisol on loess, typical and pseudogleyic Pseudogley on slope	147,2		k, fv1, kv1, n4
5	Rendzina on marl and soft limestones, calcareous Calcocambisol on soft limestones and marl, typical and luvisol Rhegosol siliceous-calcareous	633,4		P-3
15	Distric cambisol on shists, sandstones and phyllites, typical and luvisol	26,5	a1, r6	
19	Luvisol typical and akric on limestone Rendzina on soft limestones Eutric cambisol, vertic Vertisol on marl	69,7	r6, du3-4	
27	Pseudogley on slopes and plain Distric cambisol, pseudogleyic	557,4	dr2, vv	
2	Colluvial soil, calcareous, gleyic and nongleyic Eugley, calcareous and noncalcareous	346,6	N-2	r1, vv, k
8	Calcomelanosol Calcocambisol	45,7		du1, r6
32	Eugley, hypogleyic, mineral, calcareous Eugley, amphigleyic, mineral, calcareous	20,9		r4, k, vv
35	Eugley mineral Colluvial soil, gleyic Alluvial-colluvial soil	46,9		r4, k, vv
40	Eugley mineral, noncalcareous and vertic Humofluvisol alluvial Pseudogley - gley	48,4		vv, k, r4

#### Recommendations for land reclamation

Soils without major limitations for viticulture (P-1 class) are eutric cambisol, and rendzina on marl and soft limestone up to 16% slope. The main measure for their reclamation is ameliorative fertilization. Moderately suitable soil (P-2 class) are rhegosol, rendzina, anthropogenic vertisol, distric cambisol, luvisol and pseudogley on slope up to 30% slope. The main measures for land reclamation are ameliorative fertilization, humization, deep tillage and contour tillage. Soils with larger limits for growing vines (P-3 classes) are rhegosol, rendzina, vertisol, eutric and distric cambisol, calcocambisol, luvisol and pseudogley up to 30% slope. The main measures for their regulation are ameliorative fertilization, humization, deep tillage and contour tillage, terracing, drainage, calcification. Temporarily unsuitable soils for growing grapes (N-1 class) does not have, because are all used for vineyards. Permanently unsuitable soils for viticulture (N-2 class) are colluvial soil gleyic and nongleyic, eugley, calcomelanosol, very shallow calcocambisol and

pseudogley-gley. In addition to climate and topography, the main limiting factor for viticulture are periodic excess water and shallow depth of soil.

**Figure 2.:** Map of suitability of agricultural land of Zelina vineyards area for viticulture

### **Conclusions**

Area of Zelina vineyard is pedological very heterogeneous. It was found 14 different types of soil, that differ mutually in their features. Total there 4499.7 ha of soils that have different degree of suitability to viticulture (class P-1 to P-3) and 508.5 ha unsuitable soils for viticulture (class N-2). The main limiting factors for the viticulture in this area are climate (fog and frost), relief (exposure and inclination) and the soil properties (ecological depth, drainage, moisture regime, soil reaction, humus content, active lime and supply of physiologically active nutrients). For a successful intensive viticulture is necessary to carry out the following hydro and/or agrotechnical measures, individually or in combination: drainage, deep tillage, calcification, humization, ameliorative fertilization. The relief can be corrected by contour tillage or terracing.

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## THE STUDY OF LAND USE CHANGES AND THEIR IMPACT ON RUNOFF IN THE PUNKVA CATCHMENT

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### Abstract

Use of the landscape has a high influence on the water retention capacity of the soil. Water retention is important in terms of surface runoff formation. Identification of areas with increased surface runoff leads to proposals of flood protection of a catchment. The work focuses on the evaluation of factors influencing water retention in the landscape, depending on land use, based on the assessment of runoff conditions at a site in 4 periods strategically influenced by political and economic decisions leading to fundamental changes in the landscape in terms of water retention. Years evaluated were 1954, 1971, 1996, and 2009, and the site is the Punkva catchment. The area of the entire model territory, which extends into 16 cadastral areas, is 50.18 km<sup>2</sup>. The site is located in the central part of Moravia, Czech Republic.

The analysis used the existing evidence of the Czech Hydrometeorological Institute, information database on soils and aerial photos of the area. Simulation of the runoff was created in the HydroCAD model. It has been proved that the land use changes have affected the runoff. Suitable use of the landscape can reduce surface runoff from the catchment.

**Keywords:** runoff, flood protection, land use, catchment, water retention

### Introduction

Water, soil and forest – each of these takes a significant part in the creation of the landscape. They are effective in cooperation and their mutual relations are reflected mainly in the water cycle. These components of landscape do not work in their natural form – they have been modified and controlled by human activities, mainly water and forest management as well as agriculture. Based on knowledge of mutual relations and nature's principles, we can coordinate their effects by agreed regimes; collisions can be prevented and conditions supporting favourable factors can be created. The conditions of their cooperation vary in different areas: one of the components can prevail and develop at the expense of the others in some areas while in others its significance can be diminished.

Human activity considerably changes the water regime in the landscape. The effect of a functional land use on the runoff is essential and dramatic and extensive changes of land use can have a significant impact during extreme events. Deforestation, intensive agriculture, landscape urbanization and industrialization are the changes with the greatest influence on runoff processes in the landscape.

This study of changes in land use and their effect on runoff conditions has been processed for the Punkva catchment area. The area of the catchment is 50.18 km<sup>2</sup> and it extends into 16 cadastral areas.

### Materials and methods

The runoff curve number (CN) method can be used to assess the effects of various ways of catchment use, erosion prevention measures, and other changes on the surface runoff and to propose and evaluate technical erosion prevention measures. The basic input of the CN method is the rainfall. The volume of rainfall is transformed into the volume of runoff using runoff curve

numbers. The input data for the establishment of CN values included soil quality units (in Czech BPEJ). Soil quality units (SQU) represent an agronomic evaluation of sites used by agriculture based on the climate, the soil and the terrain configuration. The data for the establishment of CN values in forests came from the analysis of forest stands in the study area. Other data for the establishment of CN values were the land use in periods 1954, 1971, 1996 and 2009. The analysis of the study area with the aim to find the changes in land cover in these periods was based on aerial photography and orthophotomaps. The aerial photos were georeferenced into the coordinate system of the Unified Trigonometric Cadastral Network.

The investigation of runoff processes in the catchment also included the initial data in the form of precipitation sums and flow rates. The hydrological hydraulic model HydroCAD, in which the runoff from the catchment was modelled, used the data on rainfall when establishing the design rainfall and when deriving the intensities of calculated rainfall. The input quantity for the HydroCAD model simulation of all the researched periods was the value of two-year 24-hour precipitation sum, which is 36.5 mm for the weather station in Sloup (run by the Czech Hydrometeorological Institute). The parameters of constant intensity of calculated rain of 600 min., which enter the HydroCAD model in the form of unit hydrogram, were established using the method of reduction of daily maximum precipitation sums.

The case study also uses the vector digital terrain model 5 (DTM) created in ArcGIS, version 10, with the Spatial Analyst extension. The monitored area was divided into 16 sub-catchments, where the DTM became a basis for individual morphological features of the area, such as the hydraulic length of the catchment, the inclinations and areas of the catchment or inclinations of currents. These features were then used as input parameters for the HydroCAD model.

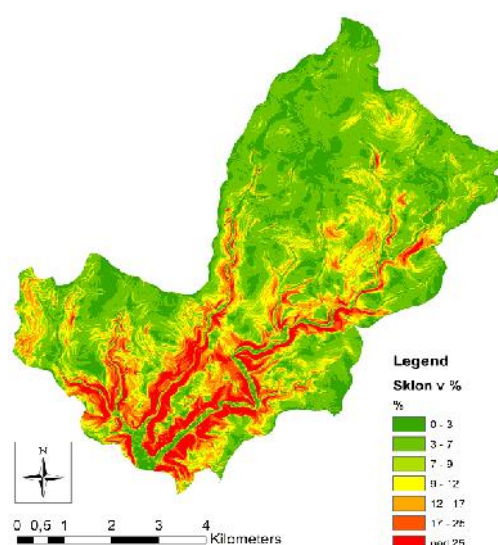


Fig.1 Study area



## Results and discussion

The georeferenced aerial photos were used as data for detailed maps of the land cover in the four explored periods. The aerial photos show the development of the landscape structure and the development of the changes in land cover. They provide an idea of the shapes, sizes and layout of lands and structural elements in the landscape as well as their changes in time.

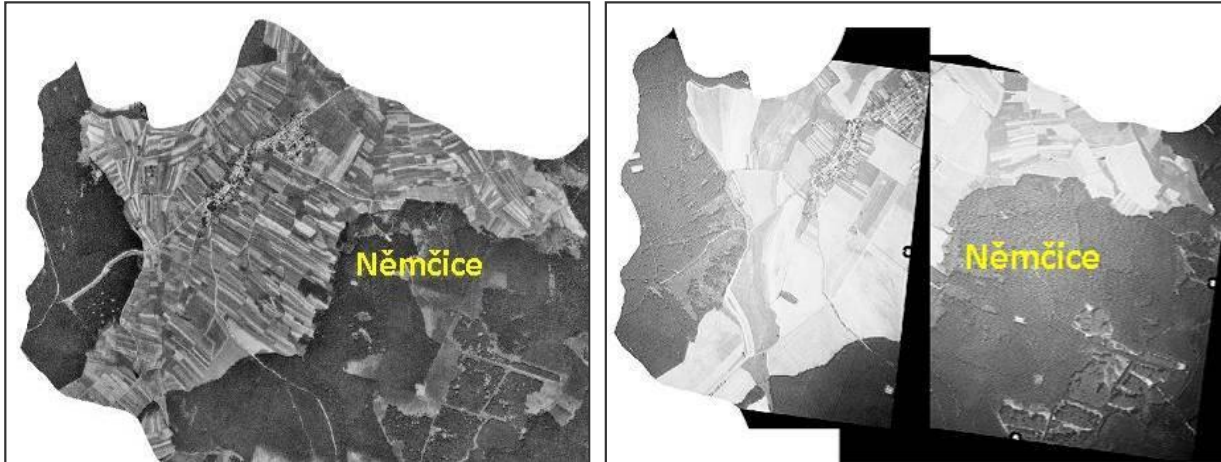


Fig.2 Examples of georeferenced aerial photos from 1954 and 1971 © CENIA, česká informační agentura životního prostředí, © VGHMÚ Dobruška

### Assessment of land cover changes

Eight categories of cover were distinguished for a detailed assessment of land use: forests, permanent grassland, arable land, scattered greenery, built-up area, water surface, gardens, and orchards. The category of permanent grassland includes meadows and pastures.

Tab. 1 Assessment of land cover changes

Land category	Land area							
	1954		1971		1996		2009	
	[km <sup>2</sup> ]	[%]	[km <sup>2</sup> ]	[%]	[km <sup>2</sup> ]	[%]	[km <sup>2</sup> ]	[%]
Forests	33.87	67.51	34.34	68.57	34.37	68.52	34.64	69.05
Permanent grassland	2.77	5.53	2.66	5.30	2.93	5.85	4.87	9.72
Arable land	12.09	24.09	11.40	22.73	10.38	20.70	8.17	16.29
Scattered greenery	0.53	1.06	0.59	1.17	0.96	1.92	0.69	1.38
Built-up area	0.63	1.26	0.81	1.61	1.18	2.35	1.41	2.81
Water surface	0.08	0.16	0.10	0.20	0.116	0.23	0.119	0.24
Gardens	0.099	0.20	0.12	0.25	0.21	0.42	0.23	0.46
Orchards	0.096	0.19	0.076	0.15	0.002	0.01	0.031	0.06
<b>total</b>	<b>50.18</b>	<b>100</b>	<b>50.18</b>	<b>100</b>	<b>50.18</b>	<b>100</b>	<b>50.18</b>	<b>100</b>

The table shows a general trend of a steady increase in the area of forests during the explored periods 1954, 1971, 1996, and 2009. Forest stands with their 34.64 km<sup>2</sup> have a dominant position in the study area. The area of forests increased by 1.54% of the total area during the observed 55 years. The area of permanent grasslands expanded up to 9.72 % in the last years. As a consequence, the area of arable lands was reduced. It decreased from 12.09 km<sup>2</sup> in 1954 to 8.17 km<sup>2</sup> in 2009, which is an 8 % decrease. A trend similar to forest stands can be seen in the built-up areas. Their area increased from 1.26% to 2.81% between 1954 and 2009.

The proportions of crop plants in the study area in the explored periods are given in Table 2 .

Tab.2 Proportions of crop plants

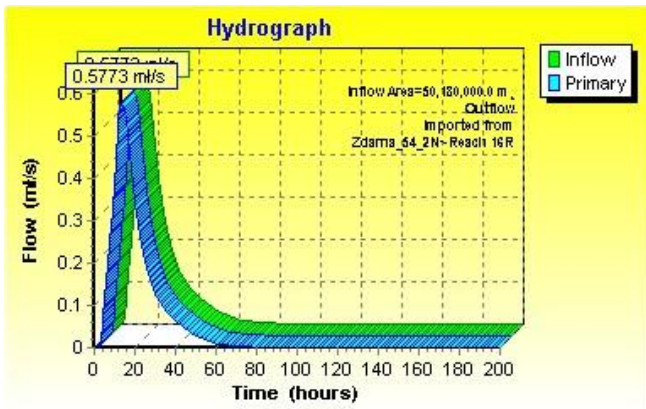
Crop plants	Area within the study area [%]			
	1954	1971	1996	2009
cereals	7.92	5.66	6.89	6.62
fodder crops	8.95	6.92	8.46	7.20
potatoes	7.22	9.23	1.46	0.05
maize	0.00	0.92	3.89	2.42
<b>arable land in total</b>	<b>24.09</b>	<b>22.73</b>	<b>20.70</b>	<b>16.29</b>

The table of crop plants shows that

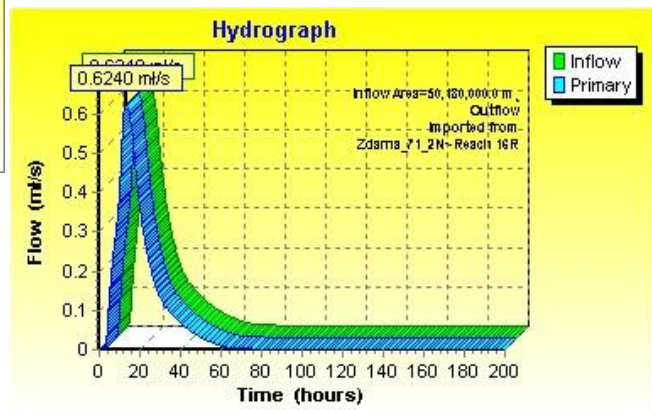
in the observed periods there is mainly a considerable change in the proportion wide-space crop plants. There has been a visible reduction in growing potatoes since the 1950s and a rise in maize since the 1970s. This has a significant impact on the runoff conditions in the study area.

**HydroCAD model outputs for periods 1954, 1971, 1996, and 2009**

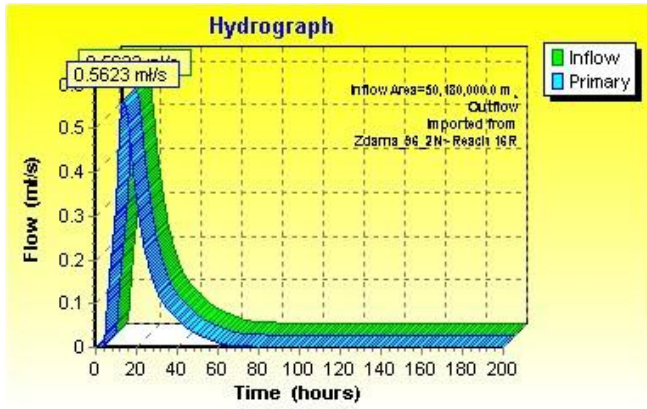
The input quantities of the model, which were variables for editing of individual periods, were the mean values of runoff curve numbers in particular sub-catchments. These values changed with the development of the changes in land cover in the given periods.



Graph 1 The result of simulation of 24-hour precipitation sum with 2- Years repetition time for 1954

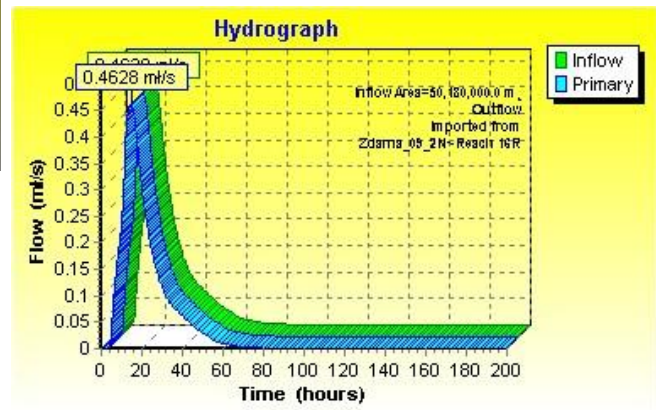


Graph 2 The result of simulation of 24-hour precipitation sum with 2-Years repetition time for 1974



Graf 4 The result of simulation of 24-hour precipitation sum with 2-Years repetition time for 2009

Graf 3 The result of simulation of 24-hour precipitation sum with 2-Years repetition time for 1996



The graphs show that the results reflect the analysed condition of the land cover in the particular years indicating the trend of a decreasing arable land area and an increasing forest area. This fact has been manifested in the simulated hydrograms. The years of the most significant changes in land use are 1954 and 1971. The results of model hydrograms from these years show an increase in culmination by 25.41%. By contrast, culmination decreased by 25.8% between 1971 and 2009. Although these values need to be considered of only an informative character due to the simplifications of input data and modelling technique, we can deduce the influence of land cover on the runoff in the catchment.

### Conclusion

The main aim of the study was to evaluate the factors affecting water retention in the landscape in dependence on land use based on the assessment of runoff conditions in the area in 4 periods strategically affected by political and management decisions leading to essential changes in the landscape as regards water retention. Considering the gained results of analyses and calculations, we can state that the data simulated for the particular periods show that a choice of plant cover can influence the runoff to a significant extent.

The changes in land use as regards land cover, size and category of lands in 1954, 1971, 1996, and 2009 significantly affected the runoff in the catchment area. The area of forest stands increased by 1.54% from 1954 to 2009. The area of permanent grassland shows an increase by 4.55% between 1971 and 2009. Also the built-up area expanded between 1954 and 2009: from 1.26% to 2.81%. There were also changes in the range of crop plants. Most considerably, there was a change in the proportion of wide-scale crops – potatoes (fodder beet, sugar beet) dominated between 1954 and 1971 and were replaced by maize starting from the 1990s.

In order to improve the runoff conditions in the study area, based on the terrain configuration and land use, it would be suitable to propose organizational, agrotechnical or biotechnical measures. As regards the organizational and agrotechnical measures, it is suitable to improve the soil structure by raising the content of humus. It is possible to use vegetation with good root systems and make the soils with a high content of clay particles lighter, which leads to the granularity of the soil profile with the optimum amount of pores. It is also possible to choose the optimum species composition

for the soil cover with a sufficient percentage of cover. By this we will achieve a higher roughness of the surface and thus also slowing down of the non-concentrated surface runoff, we will prevent destruction of the surface soil structure by the dynamics of falling rains, provide shade to the soil and thus maintain the optimum moisture for immediate water infiltration.

It is necessary to exclude wide-space crop plants (root crops, maize, etc.) from lands endangered by erosion with an inclination over 10%, or use a belt layout of the crop plants as this has a higher erosion prevention effect.

Further, it is recommendable to delineate critical areas that could be endangered by higher erosion and intensive surface runoff. The areas with an inclination over 7° should be predominantly used for permanent grassland.

As regards the biotechnical measures, measures close to nature, these are mainly erosion preventive baulks, water infiltration bands, grass sowing in valley lines, and, possibly, a polder.

The implementation of the integrated flood protection in the model area should start with area modifications of the catchment that diminish erosion effects of rainfalls and their runoff. Only then, water management and hydro technical measures can be taken using water works or other water constructions.

### Acknowledgements

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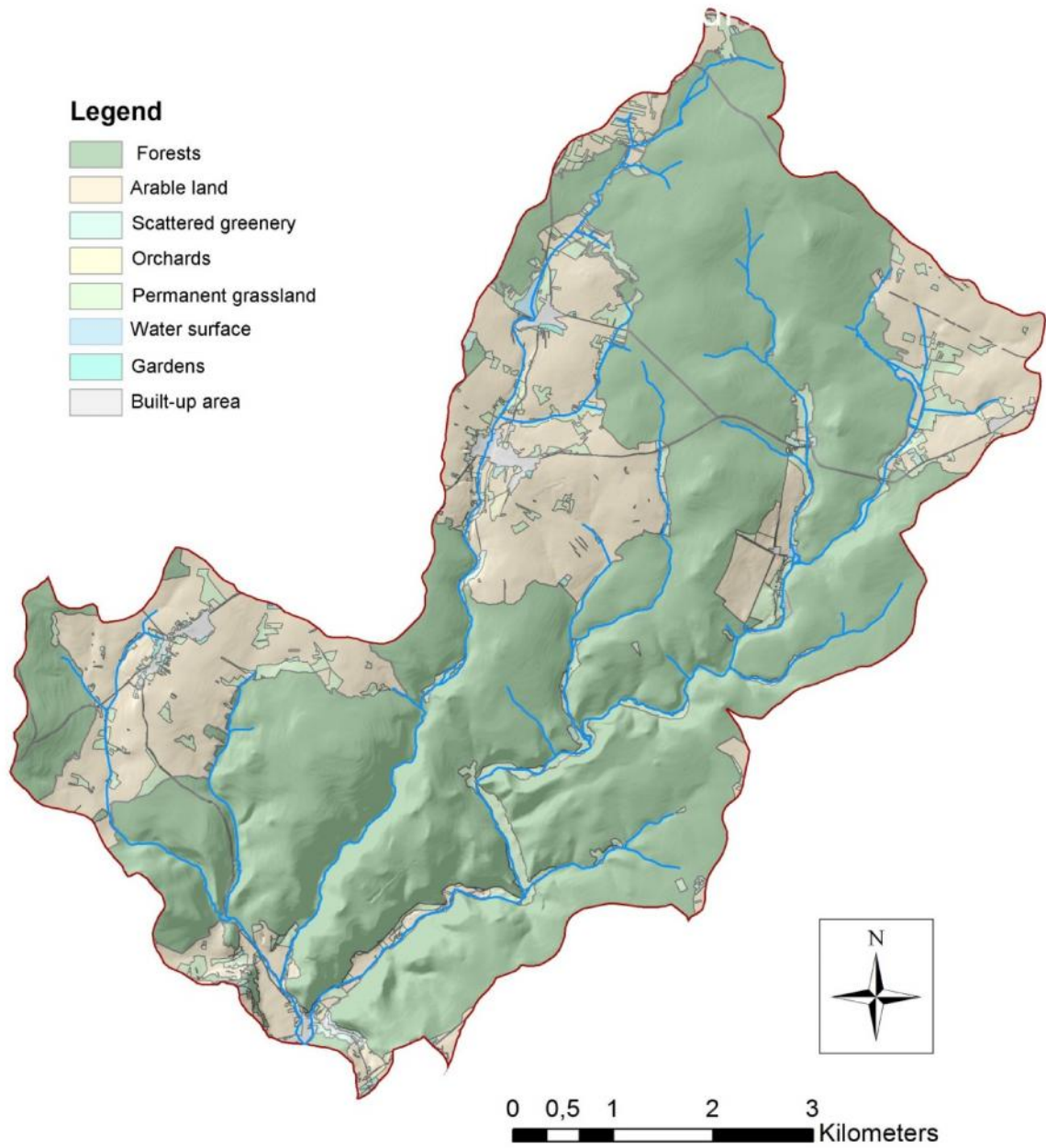


Fig.3 Land use 1954

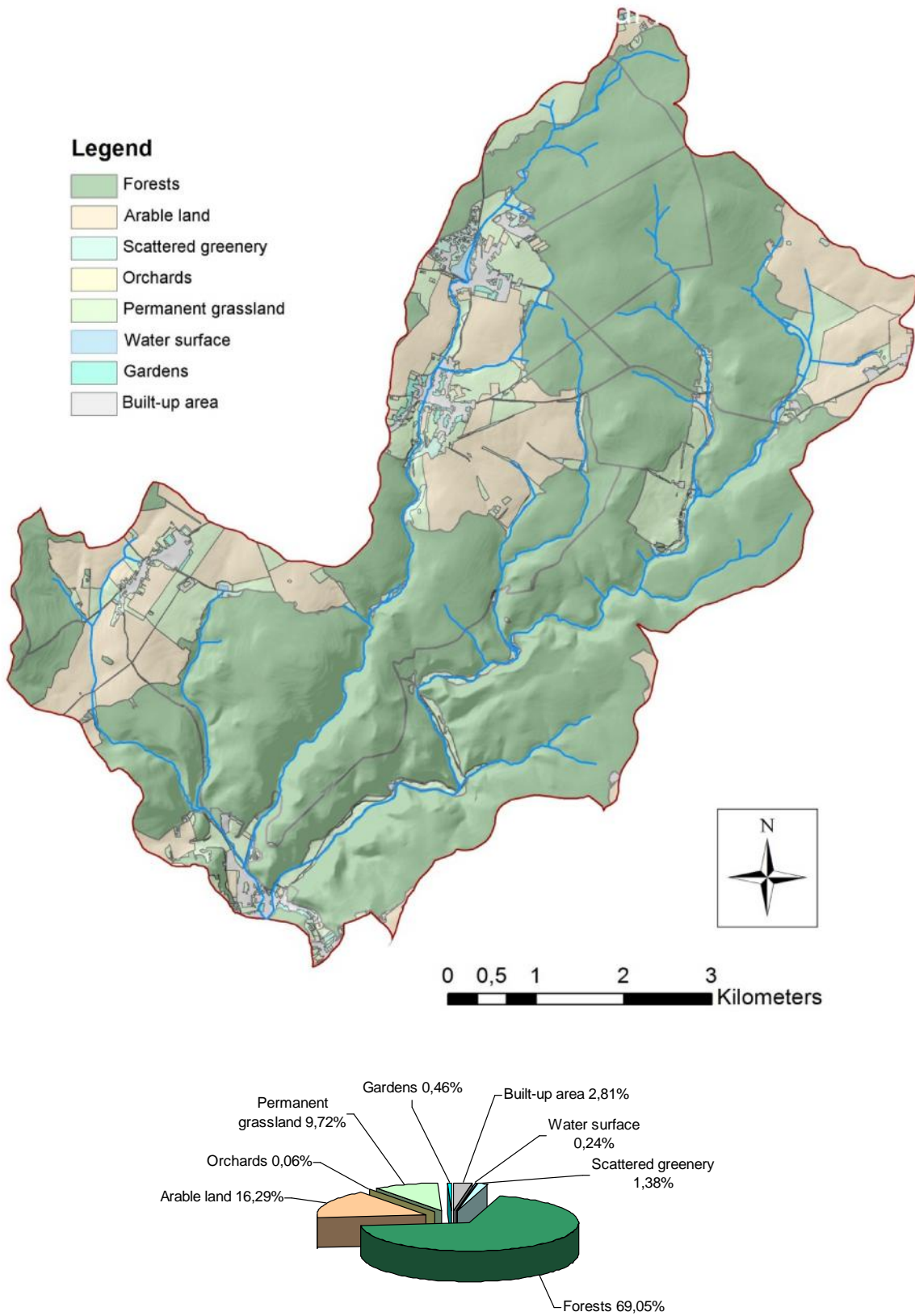


Fig.4 Land use 2009

## AGRICULTURAL UTILIZATION OF SEWAGE SLUDGE - APPLICATION TO WHEAT

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### Abstract

In a soil classified as Typic Xerochrept located in Larissa area, central Greece a field experiment was conducted to study the influence of municipal sewage sludge application on wheat yield and soil quality. The experimental design was complete randomized blocks with five treatments (control, no sewage sludge, no inorganic fertilizers-C; inorganic fertilization, no sewage sludge-IF; sewage sludge application at a rate 10 ton dry SS/ha-SS1; and 15 ton/ha-SS2) each replicated 3 times. Sewage sludge was applied at the middle of November 2009 by using an appropriate spreader. In the treatment with inorganic fertilization the conventional rates of N and P were applied (100 kg N/ha and 80 kg P<sub>2</sub>O<sub>5</sub>/ha respectively). Sewage sludge and fertilizers were incorporated in a depth 25 cm with ploughing. Wheat (*Triticum vulgare*, var. Mavragani) showing (180 kg/ha) became ten days after SS application. Wheat germination started 18 days after showing. Wheat was harvested at the middle of June, next year. Soil sampling was carried out at the showing date and at the end of July next year. The results showed that SS application increased wheat yield compared to control (from 3.78 ton/ha to 44.93 and 4.78 ton/ha in the treatments SS1 and SS2 respectively). The treatment included inorganic fertilization obtained the highest yield (5.75 ton/ha). Soil pH decreased significantly (from 8.23 in the C to 8.02 in the treatment SS2). Available P was significantly increased in the treatment SS2 (from 9.7 in C to 16 mg/kg in the SS2) but exchangeable K was not significantly affected. Nitrates concentration after the harvest was higher in the treatment SS2. No significant differences were observed in total concentrations of Zn, Pb, Ni, and Cr. It was concluded that SS application at rates 10 or 15 ton/ha may completely substitute inorganic fertilization.

**Key words:** Sewage sludge, wheat, soil quality

### Introduction

Management of sewage sludge (SS) produced by the municipal wastewater treatment plants is a serious issue for the societies. Among the various alternatives, application to agricultural land seems to be the most effective way of SS management from both economic and environmental point of view. Sewage sludge contains significant amounts of organic matter and a number of essential elements to plant growth. However at the same time SS contains also some potentially toxic substances both organic and inorganic that should be controlled in order to avoid detrimental effects to the environment if SS will be applied to the land. At European Union level about 50 % is disposed, 37% is used in agriculture, 11% is incinerated and the remaining amount is managed in various other beneficial ways (Smith, 1996). For Mediterranean environments, agricultural use of SS besides the benefit of nutrient application to the soils has an additional advantage related to organic matter enrichment of the soils that is at very low level due to several factors including climate, intensive soil cultivation and mismanagement.

To ensure the safe and beneficial use of SS in agriculture the European Union Council has adopted the directive 86/278/EEC on the protection of the environment and in particular the soil when SS is applied in agriculture (EEC, 1986) which was put in force implemented in each EU member as a relevant law. In addition, every country has each own Code of Good Agricultural

Practices through which the rules of the directive are implemented. Concerning the benefits from the agricultural use of SS, it is related mostly to the contained appreciable amount of the basic nutrients nitrogen (N) and phosphorus (P). A typical N and P content of SS is 7.5 and 3.9% respectively from which 15 and 50% respectively are considered available in the first cropping year (Smith, 1996). The availability of N is dependent on the carbon:nitrogen (C:N) ratio. The smaller the value of ratio, the higher the amount of available N. Usually, the amount of P content in SS compared to its N content is higher than that required for balanced nutrition of plants. In general, P requirement of agricultural crops is 10-25% of the N removed by the plants from soil (Cooke, 1982). So, if we estimate the amount of SS required according to the N needs of the plants we would apply considerably higher amount of P needs and we will create environmental problem. Therefore, it is better to estimate the amount of SS based on P rather than N content in order to avoid the risk of nitrate nitrogen leaching. Another important issue that should be taken into account when we apply SS to soil, is the possible harmful effects of the potentially toxic elements contained in SS. Several studies have shown that SS contain appreciable amounts of potentially toxic elements that could be uptaken by the plants (Vigerust and Selmer-Olsen, 1986). These metals may cause toxic effects to plants the sensitivity of which to each metal varies considerably. Davis and Beckett (1978) in experiments with nutrient solutions determined the upper critical concentrations of metals to barley plants above which the yield of biomass production is significantly reduced. From all those referred above, it is obvious that, if the rules imposed for SS utilization are implemented, the use of SS in agriculture may be useful. However, in order this practice to be adopted by the farmers they should become aware about the benefits of the SS use since they are usually very reservative to apply SS in their field. So far, in Greece very small amount of the municipal SS produced by the wastewater treatment plants is used in agriculture, the most important reason being for that the lack of awareness of the farmers about the benefits of the SS utilization. So, the purpose of the present study was to organize an experiment to demonstrate the people the beneficial results of the sound SS utilization in agriculture.

### Materials and Methods

In a field located near Larissa, central Greece an experiment was conducted in 2009 with the following characteristics: experimental design complete randomized blocks; treatments: control-C, no sewage sludge (SS), no inorganic fertilizers; inorganic fertilization- (IF), application of 90 kg N/ha; application of 10 ton dry SS/ha-SS1; application of 15 ton dry SS/ha-SS2; replications of each one treatment 3; plot area 0,175 ha, total area of the experiment 2,1 ha; crop wheat (*Triticum durum*, var. Mavragani); date of showing 11/2/2009. The soil of the experimental field was a Typic Xerochrept, with pH 8,3, and organic matter content 1,2-2,1%. Before showing the soil was cultivated in a depth 25 cm, sewage sludge at the rates referred above was incorporated in the plough depth and after one week the wheat was shown in a quantity of 180 kg/ha. Eighteen days after showing the emergence of the wheat plants was happened regularly in the whole showed area. Inorganic fertilizers (ammonium nitrate) was applied in the soil surface at the middle of next February. The properties of the SS applied, that came from the wastewater treatment plant of the city of Larissa, are shown in Table 1. In the same table the permissible upper of the potentially toxic heavy metals imposed by the EU are referred. Three soil samplings from each one experimental plot were done as follows: the first one just before SS application, the second at the next May and the last one next September about two months after harvest. In the samples of the first sampling the basic soil chemicals properties were determined (i.e. pH and electrical conductivity in an extract soil:water 1:1, exchangeable  $K^+$  (ammonium acetate method),  $NO_3-N$  (potassium chloride extraction method), available P (sodium bicarbonate extraction method) and the “available” amounts of the metals Pb, Ni, Cd, Cr, Zn, Cu Fe (DTPA extraction method). In the samples of the second sampling the concentration of  $NO_3-N$  was determined to monitor its evolution. Lastly in the third sampling samples, the same determinations were performed as in the samples of the first sampling, to follow



the level of heavy metals due to SS application. All the methods used are referred by Page et al. (1982). Wheat harvesting was done in the middle of the next June by an appropriate harvesting machine. Statistical analysis of the results obtained included analysis of variance performed by SPSS statistical package by using the LSD test.

Table 1. Selected properties of the sewage sludge applied (in parenthesis the upper limit values permitted by the legislation, EC 1986)

Property	Value	Property	Value, mg/kg d.w.
Organic carbon, %	28,4	Cr	65,4
Total N, %	5,02	Pb	214 (750-1200)
pH (1:10 SS:water)	6,5	Hg	4,1 (16-25)
Total P, % d.w.	2,6	Cu	264 (1000-1750)
Cd, mg/kg d.w.	1,8 (20-40)	Zn	1369 (2500-4000)
Ni, mg/kg d.w.	32,5 (300-400)	Fe	5153

## Results and Discussion

The emergence of weed seeds was started 18 days after sowing and completed in few days. It was uniform in the whole experimental area. Later, a better growth compared to control was observed to the treatments received SS or inorganic fertilization, expressed as higher height of the plants and better tillering. The color of the plants in control was lighter green than in all the rest treatments indicating inadequate nitrogen nutrition. In most replications the order of plants height was C<IF<SS1<SS2.

### Influence of sewage sludge on wheat yield

Sewage sludge and inorganic fertilizer application increased wheat yield significantly compared to control (Fig. 1). Sewage sludge at both rates resulted in lower yield compared to inorganic fertilization. This was expectable for the first year of SS application since the mineralization of organic matter needs considerable time to release available N compared to inorganic fertilizer that provides available to plants N forms very quickly. In any case it can be claimed that SS may replace inorganic fertilizers. Similar results on the influence of SS on wheat yield was reported by several investigators (Qioing et al., 2012; Motta and Maggiore, 2013)

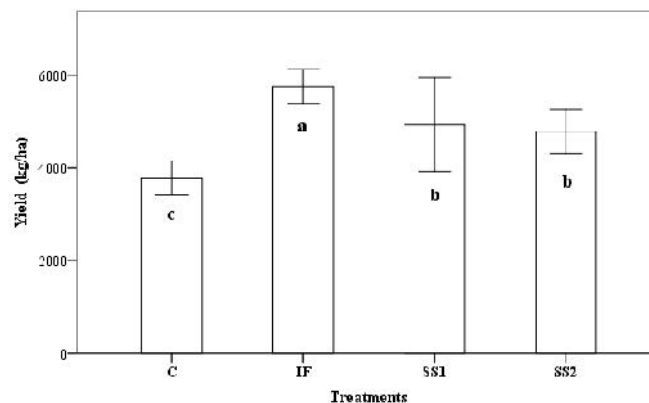


Figure 1. Influence of sewage sludge application on wheat yield (Columns with different letters differ significantly at the probability level  $p < 0.005$  according to the LSD test. The bars show the SE of the means)

### Influence of sewage sludge on soil properties

Table 2 shows the influence of SS on selected soil properties. Application of SS had limited but significant effect on soil properties studied.

Soil pH decreased by 0.2 units in the treatment received the higher SS rate. Sewage sludge may cause a decrease of soil pH due to organic matter mineralization (Tsadilas et al., 1995).

Organic matter also significantly increased in the treatment received the higher SS rate (SS2). This is important for soils with low organic matter content like the Mediterranean soils.

Available P was significantly affected by the SS application (Table 2, Fig. 2). Before its application the value of available P was around 10 mg/kg in all the experimental plots. After harvesting however, its concentration was found to be significantly higher in the treatments received SS. In the treatment SS1 its was found about 14 mg/kg and in the treatment SS2 16 mg/kg i.e it increased about 60%.

Table 2. Influence of sewage sludge application on selected soil properties and soil heavy metal content

Treatments	pH (soil:water 1:1)	Organic matter, % mg/kg	Available P, mg/kg	Exch. K, cmol/kg	Electrical Conductivity, µmhos/cm	NO <sub>3</sub> <sup>-</sup> , mg/kg
C	8,22a	1,29b	9,66b	0,40a	362	5,7b
IF	8,18a	1,54b	10,33b	0,33a	373	8,3b
SS1	8,15a	1,60ab	13,66ab	0,37a	404	9,7b
SS2	8,02b	1,87a	16,00a	0,36a	477	23,6a
	Cu, mg/kg	Zn, mg/kg	Pb, mg/kg	Cd, mg/kg	Total Ni, mg/kg	Total Cr, mg/kg
C	19,33a	40,66a	17,66a	1,03a	100,3a	97,3a
IF	20,67a	45,00a	18,33a	1,26a	104,0a	111,3a
SS1	20,33a	43,33a	16,33a	1,00a	98,00a	101,3a
SS2	20,66a	45,66a	20,00a	1,16a	106,0a	106,0a

Numbers in the same column followed by different letters differ significantly at probability level  $p < 0.05$  according to the LSD test.

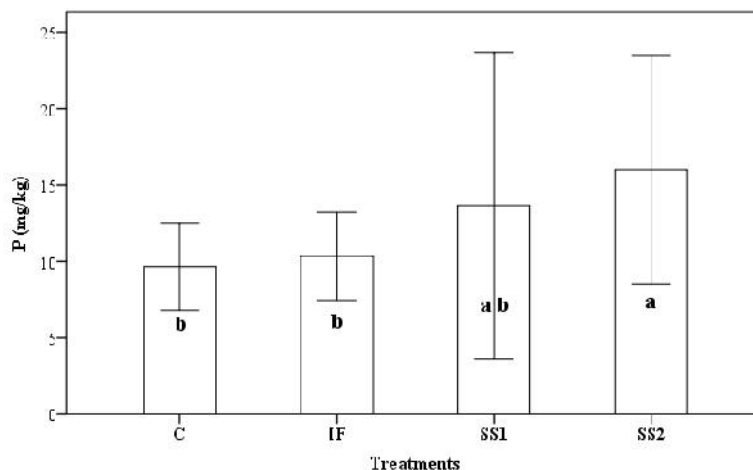


Figure 2. Diagrammatic presentation of sewage sludge application on the concentration of available soil P (Columns with different letters differ significantly at the probability level  $p < 0.005$  according to the LSD test. The bars show the SE of the means)

Concentrations of  $P_{Olsen}$  higher than 10 mg/kg are considered adequate for wheat crop (Olsen and Sommers, 1982). The contribution of SS in P is very significant since P is a non-renewable element and supplies are becoming increasingly scarce. It is estimated that the reserves of P will cover the P needs of agricultural production by 2033 and after then will face a serious shortage in P inputs (SSA, 2010). So, any possible P source such SS should be seriously considered.

Electrical conductivity (EC) slightly increased, remaining however at levels that are considered low and no restrictive for most of the crops (Marx et al. 1999).

No influence of SS application was recorded on exchangeable K as it was expected since K content of SS is low since it follows the liquid phase in the wastewater treatment process. In all treatments K concentration was found 4.0 cmol/kg which is considered low (Marx et al. 1999).

Nitrates concentration after harvesting date was found to be significantly higher in the treatment received the higher SS rate although it was at low level. Nitrates concentration was in close correlation with EC which shows that a significant part of the EC is due to nitrates ions ( $R^2 = 0.79^{***}$ , data not shown).

No one of the metals studied was significantly influenced by the SS application. This is due the low content of heavy metals of the SS used (Table 1). What is worthy to mention, is that soil Ni is quite high and according to relative directive (EEC, 1986) in soils like that application of SS is not permitted. A number of data (unpublished data of the authors) show a noticeable high concentration in Greek soils that should be examined to reveal the reasons and the possible measures to be taken.

### Conclusions

Sewage sludge from the wastewater treatment plant of the city of Larissa, central Greece is suitable properties and it can be used for substituting mineral fertilizers for wheat crop. It increases yield, enriches soil in available P and has no significant effect on soil heavy metals concentration.

### Acknowledgments

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**EFFECT OF LAND USE DISTRIBUTION PATTERNS ON NITROGEN  
CONCENTRATION IN RIVER WATERS IN AGRICULTURAL CATCHMENTS,  
WESTERN HOKKAIDO, JAPAN**

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**Abstract**

In this study, the relationship between the land uses for croplands and forests in 32 small-scale catchments in western Hokkaido, JAPAN was examined. The purpose of the study is to determine what land use distribution is beneficial for the maintenance of agricultural production and the conservation of river water quality.

River water sampling was done on 32 rivers in June, August and October 2011 and 2012, and nitrogen concentration was measured. Land-use analysis was done by using GIS, and the proportion of cropland to forestland, the spatial continuity (*SC*) of cropland and that of forestland, and the land use of riparian areas were obtained.

In catchments where the proportion of cropland is high, the nitrogen concentration in the river water is high. It was estimated that the area of current cropland would need to be reduced by up to 20% to reduce the nitrogen concentration of the river water to 1 mg/L. However, it was also clarified that decreases in the nitrogen concentration of the river water would be possible even without considerable reduction in the proportion of cropland, if the distribution of land uses was changed. Increasing the continuity of forests or changing croplands in the riparian zone to forests was found to be effective.

**Key words:** Nitrogen, River water, Upland field, Forest, Land use distribution pattern

Hokkaido's 1.15 million ha of agricultural land accounts for 25% of Japan's agricultural land, and most of that land in Hokkaido is cropland. Nitrogen used for croplands has caused river water contamination, because 50% of nitrogen input in the croplands flow out into rivers (Okazawa et al., 2003). Previous studies revealed a correlation between the nitrate nitrogen concentration in river water at normal flow and the proportion of cropland in the catchment (Ahearna et al., 2005; Muneoka et al., 2012). It can be thought that reduction in the proportion of cropland in a catchment is one way to resolve the problem of nitrogen contamination in river water. However, it is not practical to further reduce croplands. It is necessary to maintain the present level of agricultural production, because Japan is less than 50% self-sufficient in food production reported by the Ministry of Agriculture, Forestry and Fisheries, JAPAN. It has been reported that the nitrogen concentration in river water often differs in two catchments with similar proportions of cropland (Okazawa et al., 2013). The difference in the nitrogen concentration is known to be attributable to the distribution of forest areas along the river, such as riparian forest (Okazawa et al., 2010). It has been known that the presence of riparian forests changes the nitrogen concentration in river water (Lowrence et al., 1984; Hill et al., 2000); however, very few studies have further addressed this in the Eastern Asia.

**Materials and methods**

The hydrological investigation was conducted in 32 small catchments located in the Nukibetsu River basin and Shiribetsu River basin of western Hokkaido, JAPAN. In this region, the annual mean temperature is 6.1°C, and the annual low is -20.2°C(February) and the annual high is

29.7°C, (August). The annual precipitation (2012) is 1,181 mm/y, much less than the national mean of 1,700mm/y. During November to March, whole subject area has been covered by snow.

**Table 1 A summary of the surveyed catchment areas**

No.	River	Area (km <sup>2</sup> )	Land use (%)			No.	River	Area (km <sup>2</sup> )	Land use (%)		
			Cropland	Forestland	Other				Cropland	Forestland	Other
1	Pon-Nukibetu 1	10.92	41	40	19	17	No Name	11.42	32	55	13
2	Pon-Nukibetu 1	15.89	40	45	15	18	No Name	7.32	42	50	8
3	No Name	9.33	43	55	2	19	Shinosen	7.16	50	48	2
4	Oroen-nukibetu	22.14	10	75	15	20	Chiraibetu	7.42	54	40	5
5	Soutakibetu	9.48	22	72	6	21	Makkari	102.88	28	61	11
6	Soutakinetu-oku	10.82	11	63	26	22	Rubeshibe	28.09	6	74	21
7	Maruyama	4.41	3	87	10	23	Nanasi	20.23	11	65	24
8	Ponbetu	29.79	9	74	17	24	Doro	7.49	21	42	37
9	Shin-yamanashi	9.43	14	78	8	25	Inufurebetu	8.88	1	81	18
10	Ohara	6.52	48	36	16	26	Katuranosawa	11.94	0	92	7
11	Nifusinaï 2	2.01	91	1	8	27	Yahasinosawa	7.64	0	94	6
12	Nifusinaï 1	1.30	91	1	8	28	Konbu 1	9.68	1	73	26
13	Nifusinaï	13.87	79	17	4	29	Pirikanbetu	4.77	0	69	31
14	Obanai	10.55	42	52	7	30	No Name	3.44	3	47	50
15	No Name	24.39	23	67	10	31	Shintomi	6.54	25	47	28
16	Nambetu	5.90	66	30	4	32	Konbu 2	10.45	23	71	6

A summary of the surveyed catchment areas is given in Table 1. 32 catchments were chosen for this study. The proportions of cropland and forestland in each catchment area varied significantly, ranging from 0% to 91% and 1% to 94%, respectively. In all catchments, however, cropland and forested area together accounted for at least 50% of the total area, and the proportion of other land-use patterns (such as residential or industrial) was very small.

Water quality was surveyed on sunny days to avoid the effects of flood condition due to precipitation. The field survey was done six times each in June, August, and October of 2011 and 2012, respectively. Samples were taken from river water at the downstream end of each small catchment. The samples were taken back to the laboratory and their nitrogen content was analyzed using the Japanese Industrial Standard (JIS) method.

Determination of rivers and their respective catchment boundaries was made using a 1:25,000 topographic map. Additionally, a land-use map with a 100-m grid resolution, published by the Ministry of Land, Infrastructure, Transport and Tourism, was used to determine land use, such as cropland and forestland. Land-use ratios and agglomeration were analyzed using GIS software (ArcGIS 9.1, ESRI).

Three types of land-use index were used in this study;

- (1) The percentage of forested land or cropland in a catchment.
- (2) Using the buffering function of ArcGIS software, we set buffer zone demarcated from the channel centerline outward ( $BZ_{100}$ ). The subscripts indicate the width of the zone in meters, with the riverbank as 0 m (Okazawa et al., 2010). The ratio of percent of forestland in  $BZ_{100}$  (km<sup>2</sup>) to the percent of cropland in the catchment (km<sup>2</sup>) was defined as the Land Use Index ( $LUI$ ), and its relation with nitrogen concentration was investigated.

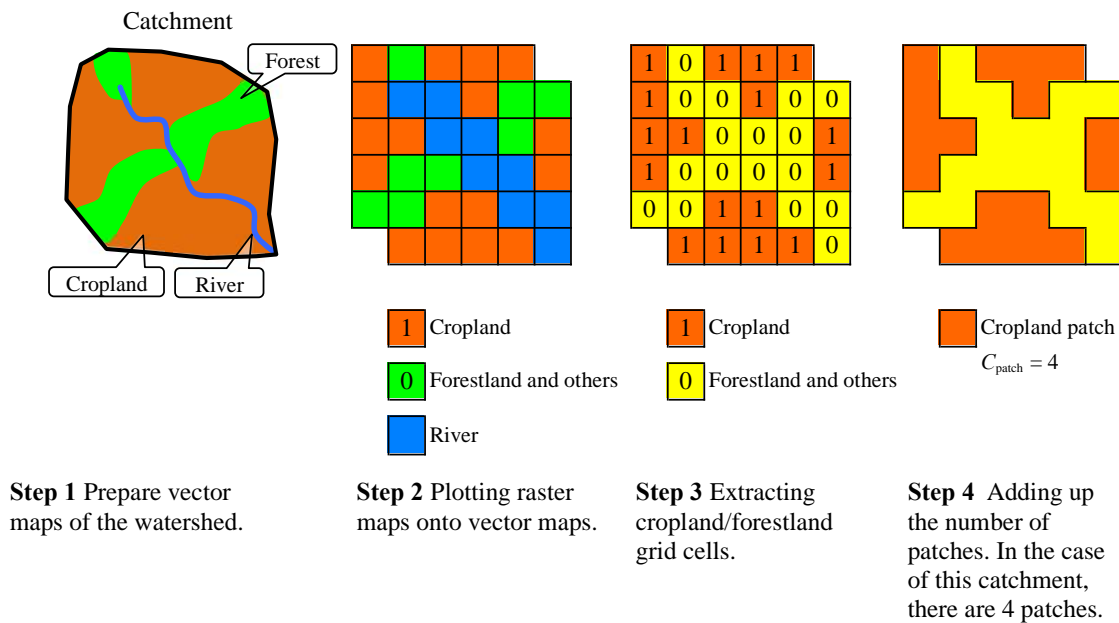
$$LUI = \text{Forest area in } BZ_{100} / \text{Cropland area in the catchment} \quad \text{Eq. 1}$$

- (3) Indices for the land-use agglomeration include those that show how “joined” or “clumped” land-use areas are, as well as the “spatial continuity” ( $SC$ ) index of identical land use. In this study,  $SC$ , which can be simply derived from GIS, is employed as the index for land agglomeration.  $SC$  was proposed by Tsunekawa et al. (1991) and Okazawa et al. (2011). A land-use map with a grid resolution of 100 m was used to calculate  $SC$ . An example of  $SC$  calculation for cropland is shown

in Fig. 1. To obtain an *SC* index, grids representing cropland were grouped into “patch” units. A patch refers to a group of grid cells connected in a vertical, horizontal, or oblique direction. In Fig. 1, the cropland grid cells are grouped into 4 patches. After all subject grids were grouped into patches, *SC* was obtained using Eq. 2.

$$SC = k / C_{\text{patch}} \tag{Eq. 2}$$

where *k* is the number of grid cells representing the cropland area in a catchment, and  $C_{\text{patch}}$  is the number of patches representing groupings of these cells. In the example given in Fig.1, the number of grid cells representing cropland, *k*, is 17, and  $C_{\text{patch}}$  is 4, so *SC* is 4.25. In other words, *SC* is equivalent to the average area of a patch in each catchment. The *SC* for forested land was calculated using the same method.



**Fig.1 Land use and attribute values**  
(  $k = 17$ ,  $C_{\text{patch}} = 4$ ,  $SC = 4.25$  )

### Results and discussion

The nitrogen concentration in the river water shown in Table 2 for each catchment is the mean and standard deviation of measurements obtained by six samples. The minimum and the maximum T-N concentration are 0.14 mg/L, 4.29 mg/L, respectively. T-N contamination in river water exceeding 1 mg/L, which is the environmental standard value, was found in the sample at 15 out of 32 locations. In most catchments,  $\text{NO}_3\text{-N}$  accounted for a large portion of the T-N.

Fig.2 shows relationship between percent of cropland in each catchment and concentrations of T-N in the rivers. The higher is the percent of cropland, the higher are the concentrations of T-N. The correlation between the percent of cropland and the nitrogen concentration is very strong ( $r^2=0.84^{**}$ ). This suggests that such agricultural land use contributes greatly to the variation in the nitrogen concentration in river water. It was also clarified that, in order to lower the nitrogen concentration in river water to within 1 mg/L, which is the environmental standard value in Japan, or lower, it was necessary to reduce the area of cropland to about 20% of the catchment.

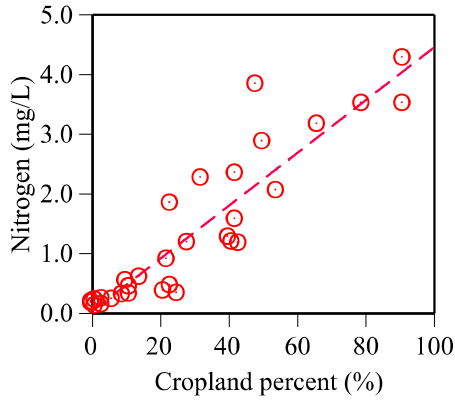
Fig.3 shows the relationship between *LUI* and T-N concentration. T-N concentration decreased with increases in *LUI*. A negative correlation was obtained between *LUI* and T-N concentration. The above findings show that, in order to lower the nitrogen concentration in river

Table 2 Nitrogen concentration of investigated rivers

No.	①T-N(mg/L)		②NO <sub>3</sub> -N(mg/L)		NO <sub>2</sub> -N(mg/L)		NH <sub>4</sub> -N(mg/L)		Org-N(mg/L)		②/①
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
1	1.21	0.29	0.81	0.15	0.00	0.00	0.04	0.01	0.36	0.35	0.67
2	1.29	0.25	0.90	0.07	0.01	0.01	0.05	0.03	0.33	0.32	0.69
3	1.19	0.25	0.91	0.03	0.01	0.00	0.05	0.01	0.23	0.25	0.77
4	0.56	0.16	0.28	0.08	0.00	0.00	0.03	0.02	0.25	0.24	0.50
5	0.92	0.57	0.57	0.54	0.01	0.02	0.04	0.03	0.29	0.27	0.62
6	0.34	0.17	0.12	0.14	0.00	0.00	0.03	0.02	0.19	0.13	0.34
7	0.26	0.15	0.06	0.06	0.00	0.00	0.03	0.01	0.16	0.20	0.25
8	0.33	0.11	0.15	0.06	0.00	0.00	0.03	0.02	0.14	0.09	0.47
9	0.62	0.21	0.40	0.21	0.01	0.02	0.07	0.05	0.14	0.16	0.65
10	3.85	2.39	1.03	0.40	0.16	0.07	1.04	1.13	1.63	1.60	0.27
11	3.53	0.94	2.92	0.63	0.02	0.01	0.08	0.08	0.51	0.54	0.83
12	4.29	0.29	3.94	0.65	0.02	0.01	0.03	0.03	0.30	0.40	0.92
13	3.53	0.08	3.27	0.30	0.01	0.01	0.03	0.03	0.23	0.24	0.93
14	1.59	0.17	1.26	0.28	0.01	0.01	0.03	0.02	0.30	0.14	0.79
15	1.86	0.45	1.51	0.44	0.01	0.01	0.07	0.03	0.28	0.29	0.81
16	3.18	0.72	2.89	0.56	0.01	0.01	0.05	0.01	0.24	0.24	0.91
17	2.28	0.24	1.81	0.18	0.01	0.01	0.03	0.02	0.42	0.40	0.80
18	2.36	0.24	1.82	0.23	0.01	0.00	0.05	0.01	0.47	0.16	0.77
19	2.89	0.79	2.54	1.07	0.01	0.00	0.03	0.03	0.31	0.40	0.88
20	2.07	0.54	1.59	0.23	0.00	0.00	0.02	0.02	0.46	0.38	0.77
21	1.20	0.12	0.96	0.06	0.01	0.00	0.03	0.01	0.19	0.06	0.80
22	0.25	0.08	0.11	0.05	0.00	0.00	0.03	0.01	0.11	0.04	0.42
23	0.46	0.08	0.31	0.07	0.00	0.00	0.04	0.02	0.11	0.03	0.67
24	0.39	0.10	0.22	0.04	0.00	0.00	0.02	0.01	0.14	0.05	0.57
25	0.14	0.03	0.04	0.01	0.00	0.00	0.03	0.03	0.07	0.06	0.31
26	0.19	0.04	0.11	0.03	0.00	0.00	0.02	0.01	0.06	0.02	0.59
27	0.19	0.02	0.08	0.03	0.00	0.00	0.03	0.02	0.08	0.02	0.43
28	0.24	0.05	0.08	0.03	0.00	0.00	0.05	0.04	0.11	0.05	0.34
29	0.21	0.07	0.11	0.03	0.00	0.00	0.02	0.00	0.07	0.06	0.55
30	0.16	0.05	0.06	0.02	0.00	0.00	0.03	0.03	0.08	0.01	0.35
31	0.35	0.05	0.23	0.09	0.00	0.00	0.03	0.03	0.09	0.03	0.65
32	0.48	0.14	0.16	0.06	0.01	0.00	0.04	0.04	0.27	0.12	0.34

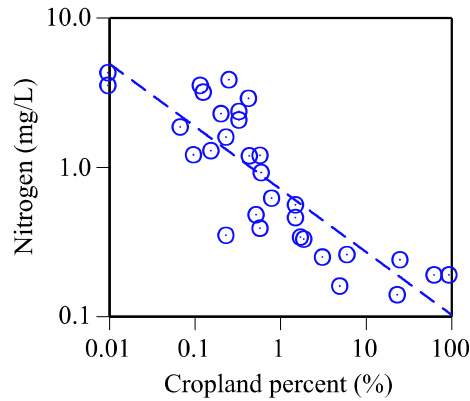
water, it is effective to increase forestland within *BZ*<sub>100m</sub> or to reduce the overall area of cropland in the catchment.





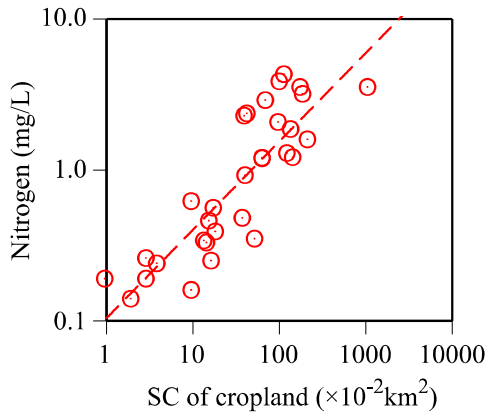
$$y = 0.044x + 0.040 \quad (r^2 = 0.84^{**})$$

Fig.2 Relationship between T-N and cropland percent



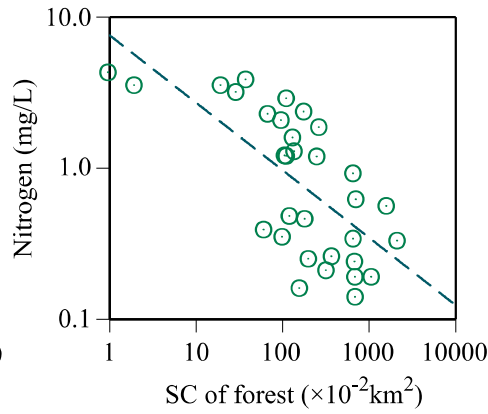
$$y = 0.71x^{-0.41} \quad (r^2 = 0.71^{**})$$

Fig.3 Relationship between T-N and LUI



$$y = 0.10x^{0.59} \quad (r^2 = 0.74^{**})$$

Fig.4 Relationship between T-N and SC of cropland



$$y = 7.6x^{-0.45} \quad (r^2 = 0.47^{**})$$

Fig.5 Relationship between T-N and SC of forestland

Fig. 4 and 5 show the relationship between *SC* and T-N concentration. Positive correlation with 1% significance was found between the *SC* of cropland and T-N concentration. Negative correlation with 1% significance was found between the *SC* of forests and T-N concentration. Based on the above examination, it is thought to be possible to lower nitrogen concentration in river water by only decreasing the patch area of cropland or by increasing the patch area of forests without changing the total areas of cropland or forest in a catchment.

### Conclusion

It was determined that the following land-use options would be effective in lowering the nitrogen concentration in river water.

- 1) Reducing the area of cropland to about 20% of the total catchment area; however, this reduction lowers agricultural productivity.
- 2) Increasing forests along the river without decreasing the area of cropland in the catchment. Reducing the patch area of the cropland. It is possible to lower the nitrogen concentration in river water by increasing the patch area of forests.

In Japan, forests are mainly owned by the national government and agricultural land by farmers. Agreement of the land owner is necessary in order to change the land use. Based on the

above described conditions, it was determined that installation of riparian forests, in which land-use change and reduction in the area of cropland are minimum, is the most effective means for conserving river water quality.

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## AGROFORESTRY - POSSIBILITIES OF MULTIFUNCTIONAL LAND USE

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### Abstract

The paper aims to point out the importance of applying the methods and technologies of agroforestry in the countries of the region. This is a practice that has been used with great success throughout the world, in different climatic zones. The advantages are numerous – besides increased crop production, benefits from properly designed and managed agroforestry practices include: economic gain, healthier environment, soil conservation and improved soil quality, sequestration of atmospheric carbon, increased biodiversity, improved landscape and many others. Though the possibilities for multifunctional land use are numerous, benefits large and quality of plants grown in association with trees often improved, in Serbia and other countries in this region agroforestry is not implemented enough. Some of the agroforestry components are separately applied in some parts of the region, e.g. wind protection belts, planting of soybeans or corn in the poplar plantations of E.A. cultivars, pig's nutrition in the forests of oak or beech. Methods and technologies of agroforestry that can be applied in temperate regions are much more numerous and this paper discusses some of them.

**Key words:** agroforestry, land, multifunctional use, benefits

### Introduction

Agroforestry is not a new methodology or technology. It has a very long (although often forgotten, or with a different name) history - from man's earliest attempts at agriculture to the present time. Since the Greek and Roman Antique time, when olive trees contributed to the culture of wine, cereals and legume, it is one of the most integrated and intensive approach to agricultural production system that includes trees and shrubs as a very important component to achieve environmental, economic and social goals. Traditional agroforestry has progressively been replaced by more simplified production systems. Agroforestry has been modernized over the last years. It is no longer focused on single species plantation. It is now a side by side cohabitation of local field and forest species.

Agroforestry systems have often been neglected in Europe because administrative structures within many national governments have considered that only agriculture or forestry is legitimate within their remit. This has resulted in the loss of agroforestry systems in European countries and an impoverishment of the benefits that they provide (McAdam *et al.*, 2008). But today, agroforestry becomes the part of the EU culture. This system can play a key role in meeting today's needs such as food security, climate change mitigation, water storage and purification as well as biodiversity preservation etc.

The integration of agriculture, farming and forestry can provide numerous benefits, because the land can simultaneously be used for many purposes and also make easier the transition from one type of crop to another as market demand.

But, agroforestry does require more planning than simpler land uses, because it must be taken into consideration the diverse needs of each component. In agro-forestry, combinations of trees, crop and livestock are designed and managed as a whole unit. In order to enhance the agricultural production, the biological and physical interaction between the crop and the livestock components are managed.

#### **Agroforestry in Serbia**

In Serbia, agroforestry is not applied systematically, organized and on large areas, but sporadically, intuitive and in smaller households. Some of the agroforestry components are separately applied in some parts of Serbia. Thus, for example, wind protection belts are very intensively and successfully applied in the province of Vojvodina, impacting significantly on increasing the quality and yield of crops. In order to obtain more stable ecosystems in Vojvodina and primarily prevent the removal of fertile topsoil by wind erosion, there is a great need to increase the network of forest windbreaks, which would also increase the current forest cover. By measuring the change of wind speed at several points in front of the belt, in the belt, and behind the belt, there were obtained values which indicate the effectiveness of the belt in reduction of wind speed.

Besides windbreak shelterbelts, in Serbia is sporadically applied planting of soybeans or corn in the poplar plantations of E.A. cultivars. Soybean intercropping increases soil microbial biomass, microbial substrate use efficiency, and soil N fertility. Soybean intercropping also results in higher aboveground biomass increment and higher soil N response efficiency of hybrid poplar trees.

Formerly more often used form for pig's nutrition in the forests of oak (acorns) or beech (bechnuts), now is again returned to practice, especially for the old pig breeds such as “Mangulica”.



Photo 1. Grazing system which includes natural resources of pasture and oak woodland

The similar way of pig breeding is applying in Croatia with Black Slavonian “Pfeiffer” breed. Traditional Black Slavonian pig production is an outdoor, grazing system which includes utilization of natural resources of pasture and oak (*Quercus robur* L.) woodland with supplement of a small amount of corn seed or some other grains (~0.15 kg per head daily) (Karolyi *et al.*, 2010).

#### **Benefits of agro-forestry**

Research over the past 20 years has confirmed that agroforestry can be more biologically productive, more profitable, and be more sustainable than forestry or agricultural monocultures.

Temperate agroforestry systems are already widespread in many parts of the world, due to the fact that they can: increase crop yield, reduce soil erosion (losses of water, soil material, organic matter and nutrients), maintain soil fertility, some tree and shrub species can increase nitrogen in soil, decomposition of tree litter and prunings can also contribute to soil fertility, reclaim polluted soils, utilize solar energy more efficiently than monocultural systems, lead to reduced insect pests and diseases, moderate microclimates, create a healthier environment, enrich biodiversity and improve

landscape, provide a more diverse farm economy and stimulate the whole rural economy, leading to more stable farms and communities. Economic risks are reduced when systems produce multiple products.

Benefits of agroforestry are still insufficiently known, but they are numerous and significant.

### **Methods and technologies of agroforestry that can be applied in temperate regions**

#### Windbreaks (also: shelterbelts, wind protection belts)

Rows of vegetation - linear plantings of trees and shrubs designed to reduce and redirect wind, enhance crop production, protect people and livestock, and benefit soil and water conservation.

Crop yield increases in fields adjacent to shelterbelts have been reported in many studies. These increases occur because of improved microclimates and better moisture retention, reduced wind speeds and thus reduced wind erosion and damage to crops. *Field windbreaks* protect a variety of wind-sensitive crops, improve crop yields and water-use efficiency, reduce wind erosion, and increase bee pollination and pesticide effectiveness. *Livestock windbreaks* help reduce animal stress and mortality, reduce feed consumption, and help reduce visual impacts and odors. *Living snow-fences* keep roads clean of drifting snow and increase driving safety. They can also spread snow evenly across a field, increasing spring soil moisture. *Farmstead windbreaks* enhance living and working environments, add value of home and help conserve energy.

The effectiveness of windbreak forest belts depends significantly from their density, height, width, permeability and choice of tree and shrub species.

As a rule, wind protection belts of tall, long-lived trees combined with shrub species, planted in rows perpendicular to the prevailing winds direction provide the greatest yield increases. In the case of areas where the direction of the prevailing wind it is not clearly defined, the belts can be built on all sides the fields in the rectangle or square form. In addition to its function to reduce wind speed, wind protection belts also reduce the evaporation from the soil and transpiration. They also affect on the balanced distribution of the snow cover preventing its removal, and increase the soil moisture. The fields that are protected by belts have higher soil moisture, and also higher air humidity. Crops differ in their responsiveness to shelter. Winter wheat, barley, rye, alfalfa and hay are highly responsive to protection, while spring wheat, oats and corn respond to a lesser degree.

Well planned shelterbelts can provide many benefits to livestock during winter and summer as well as screening noises, unsightly areas from neighbors, roads, and living areas and filter dust from roads. Odors can be diffused by properly placed shelterbelts and some odors can be absorbed onto the trees within the shelterbelt. They protect livestock from wind chill in winter and provide shade in the summer. This protection lowers animal stress and diseases, increases feeding efficiencies. There is also snow control and energy savings for heating during the winter period and cooling during the summer period.

#### Silvoarable (intercropping and alley cropping)

This is practice where trees are grown in rows with wide alleys in-between for cultivating crops. In this way, agricultural or horticultural crops are grown simultaneously with a long-term tree crop to provide annual income while the tree crop matures. Overwintering crops (i.e. autumn-sown) are very efficient users of the almost full light available over the dormant season of deciduous trees

Benefits from this method are numerous: wood or tree products are produced in addition to agronomic crops, with no reduction in crop yields. Crop quality and yields can be even increased by enhancing microclimatic conditions and increasing of soil nutrients. Biodiversity is enriched, wildlife habitat and corridors are created and aesthetic of landscape is improved.

#### Forest farming

The usual definition of Forest Farming System (FFS) implies that it is “*a distinctive approach to land management that combines management practices of conventional forestry with those of*

*small-scale farming or gardening to attain an environmentally and economically sustainable land-use system.*” Most often, FFS is established by thinning an existing woodland or plantation to leave the best canopy trees for continued timber production and to create the appropriate conditions that favor the overtopped crop. It's not just a question of self-increase profits, but also special pleasure collecting or growing non-timber forest products as part of their cultural and family tradition. Many high-value specific crops, cultures and forest products can be cultivated or grown under the protection of existing forests.

It is a way of utilizing forests for short-term income while high-quality trees are being grown for wood products. The amount of light in the stands is altered by thinning, pruning, or adding trees. Existing stands of trees can be intercropped with annual, perennial, or woody plants. The most commonly are grown five main categories of crops: food - mushrooms, nuts, vegetables, honey from bee plants, herbs, fruits (blueberries, elderberries, blackberries, raspberries, strawberries, etc.), edible flowers, sap products (e.g. maple syrup), then botanical products (medicinal plants, ornamental (decorative plants), handicrafts (willows for basketry materials), short-term energy coppice and wood products (charcoal, fuelwood, etc.). Areas used for forest farming are usually small, and systems usually focus on a single crop plus timber, but can be also designed to produce several products. Economic benefits of forest farming can be significant.

#### Silvopasture

These systems comprise trees or shrubs deliberately introduced into a forage production system, the whole designed to produce a high-value tree component, while continuing to produce the forage and livestock component indefinitely or for a significant time. Deciduous trees (fuelwood, fruit crops as tree component, chestnuts, and hazelnuts) are more readily browsed than conifers. Trees can be planted at wide spacing, in rows with forage alleys between, or in clusters. Trees provide shade and wind protection, which reduce heat stress and wind-chill of livestock, so performance is improved and mortality reduced.

The combination of tree and pasture production has been recently promoted by the EU. Pasture production under trees produces annual farm outputs which promote long term rural population stabilization compared with exclusively forest systems.

#### Forest gardening (home gardening)

Designed agronomic system based on trees, shrubs and perennial plants. These are mixed similarly to the structure of a natural forest - the most stable and sustainable type of ecosystem in specific climate. These systems are biologically sustainable, productive and require low maintenance. The crops which are produced include fruits, nuts, edible leaves, spices, medicinal plant products, poles, fibers for tying, basketry materials, honey, fuelwood, fodder, mulches, game, and sap products. A large number of species used, gives great diversity and the careful inclusion of plants increase fertility of soil (f.e. nitrogen fixers).

A forest garden is organized in up to seven ‘layers’: Canopy trees - the highest layer of trees, small trees and large shrubs, mostly planted between and below the canopy trees, shrubs (mostly shade tolerant), herbaceous perennials, ground covers, climbers and the final ‘layer’ is the root zone or rhizosphere.

Forest gardens provide a long-term biologically sustainable system for growing food and other products for a household, requiring little work for maintaining.

### Riparian forest buffers

Riparian forest buffers represent vegetation strips that physically separate agricultural land or pasture from water bodies. The main task of these protection belts, consisting of trees, shrubs, herbaceous plants and grasses is to prevent conflicts that may arise between agriculture or animal husbandry, and protection of water resources. Agriculture and animal husbandry are serious sources water contamination because they generate non-point source pollution. Plants in riparian forest buffers have the ability to filter and capture sediment, absorb and store nutrients, heavy metals and other pollutants that would otherwise enter the waterways. In addition, they prevent erosion of water banks and surrounding land through stabilization of substrates by their root system. Riparian forest buffers also act as a physical barrier that can prevent or reduce over-the-bank flood waters. These vegetation strips create suitable habitat for a variety of aquatic and terrestrial animals. Large trees on the banks form a shadow on the water surface and thus regulate the temperature of the water. Such trees and shrubs also provide birds and animals with food, shelters and nesting places. Riparian forest buffers are significant travel corridors for wildlife. Typical construction of riparian forest buffers can be seen in figure 1. The first zone (nearest to water) usually represents a narrow section of natural forest vegetation adapted to flooding conditions (AFTA, 2012). The primary role of trees and shrubs in this part of the belt is to provide stabilization of banks, a suitable habitat for aquatic plants and animals, and act as the last barrier for the filtration of substances that are removed by passing through the buffer. The second zone, which usually consists of fast-growing trees and shrubs adapted to periodical flooding conditions (AFTA, 2012). The primary role of vegetation in this zone is uptake, storage and decomposition of sediments, nutrients and other non-point source pollutants. The third zone is adjacent to agricultural fields or pastures and consists of grass and meadow vegetation (AFTA, 2012). It provides a high degree of infiltration, sediment removal and uptake of nutrients and other substances and facilitates in converting concentrated flow to sheet flow.

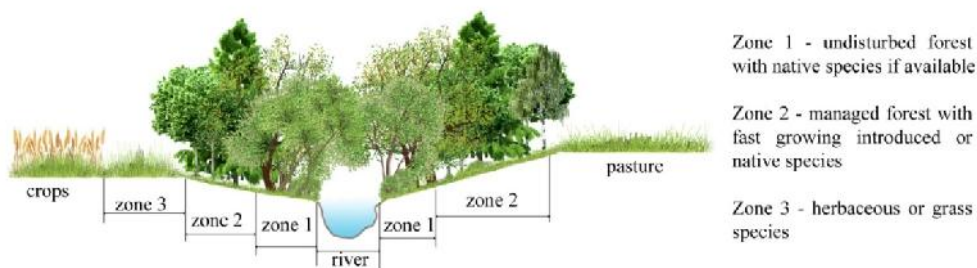


Figure 1. Different zones of riparian forest buffers (orig.)

### Conclusions

Though implementation of agroforestry could considerably contribute to the improving of agricultural production and the quality of life of the population in the areas of with moderate-continental climate, it is insufficiently applied in the countries of this region. Besides the mentioned, benefits are large and numerous. In the sphere of environmental protection, trees and shrubs contribute the air, soil and water quality. The quality of plants grown in association with trees is often improved. Agroforestry practices can improve both terrestrial and aquatic habitat, as also landscape characteristic. Populations of many wildlife species often increase with the addition of trees and shrubs into agricultural areas, and this increase provides opportunities for recreational uses. Agriculture, especially those related to protected areas, as well as agroforestry in areas with high natural value are of great importance for the conservation of biodiversity.

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## THE QUALITY OF SPRING WATERS OF FRUSKA GORA (VOJVODINA)

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### Abstract

During the period from September 2011 to February 2012, the monitoring of spring, surface and groundwaters of Fruska Gora on 8 permanent and 15 temporary points for sampling was carried out. The water points on the three sources in the Old Ledinci and Sremski Karlovci were selected for sampling. The spring waters were established to have increased contents of: orthophosphates (0.27-0.5 mg/l), phenols (0003-0118 mg/l) and boron (0.5 mg/l). These parameters were above the MAC (regulated by law) for the use of water for drinking, bottling as well as in the production of consumable fish ponds. The samples were taken in Sremski Karlovci in the period from 2003 to 2012.

**Keywords:** orthophosphates, phenols, springs

### Introduction

Fruska Gora is located easternmost within the chain of horst Slavonian mountains. It belongs to comparatively low mountains, because its highest peak Red Cot barely exceeds 500 m. Morphologically, the mountain stretches east-west being 80 km lengthwise and up to 15 km widthwise. It has an elongated lens-like shape and covers an area of 500 km<sup>2</sup>. Its northern border is the River Danube, while the southern boundary is approximately the contour of 100.00 m above sea level, stretching along the line of Sid-Erdevik-Irig- Maradik-Krcedin-Old Slankamen.

This paper presents some preliminary results for spring water on the northern slopes of Fruska Gora. Water samples were collected from the Lazin water springs near the village Ljubi in the western part of Fruska Gora, then from the water springs in the Old Ledinci on the northern slopes as well as from those of Mutica and Varadinska fountain in Sremski Karlovci.

### Materials and method

Throughout the annual survey, 12 observation points for field and laboratory tests were determined. The basic unit for the reinterpretation of existing documentation and setting up observation points network was the basin topography and hydrogeology. In terms of their type of porosity, sediment genesis and manner of occurrence in the basin, the water sources could be divided into the spring, surface and ground water (Stojiljkovi , 2003; 2004a; 2004b; 2005a; 2005b).

When interpreting the results, the longer time series of various parameters of the chemical composition of spring water were used, the better they enabled the use of the statistical method. Its program STATISTICA, version 10, was used.

At the western, northern and eastern slopes, the points on the cadastral number 4, 6 and 8 (Fig. 1, 4), were set. The two complete and two shortened (only for some micro-components and organic matters) analyses were made (Table 1).

Laboratory testing of the water was performed by a verified laboratory of the Institute of Public Health of Vojvodina-Centre for Hygiene and Human Ecology in Novi Sad. Water quality was defined in relation to the criteria for the quality of drinking water, bottling and fish production (Stojiljkovi , 2012; Zrni , 2005).

### Results and discussion



Figure 1. Geographical position of the point 4 in Kamenarski stream in Ledinci

The spring under the cadastral number 4 in the Old Ledinci, was tapped on the left side of the stream. Directly above the intake structure, a residential building with outbuildings and pigsties, was located. The results of physico-chemical testing of the spring waters, are given in Table 1.

**Physical properties:** Coverage sources ranged from 0.38 l/s-0.33 l/s, the water was colourless, odourless. The water temperature was 11.2-12.8°C, pH index of the test water 7.2-7.4 showing a neutral to alkaline medium. The values of the hardness were found to range from 24 to 24.8°dH. According to Klute- this classification of water includes "hard" waters. Mineralization was determined through the values of dry residue obtained analytically and being quite variable in the samples. Thus, the values of dry residue ranged from 683.5- 699.4 mg/l. Conductivity was considerably high, ranging from 1061-1108 µS/cm, exceeding the MAC for drinking water.

**Chemical composition:** Based on the investigation results, the chemical composition of the ground water from the spring denoted to the water being analysed to fall under the hydrocarbon class, calcium-magnesium-sodium group (according to the classification Alekin-a). Thus, within the cation content, the cation composition of calcium ions were reported to prevail with 37.8% eq. on average. The content of magnesium ions was found to amount to 36.8% eq. and that of sodium ions to 25.3% eq. on average (Fig. 2, 3). The anion content was absolutely dominated by hydro-carbonate ions, amounting to 82% eq. on average. The total content of sulfate ions was 7.8% eq. and that of chloride ones 10.1% eq.

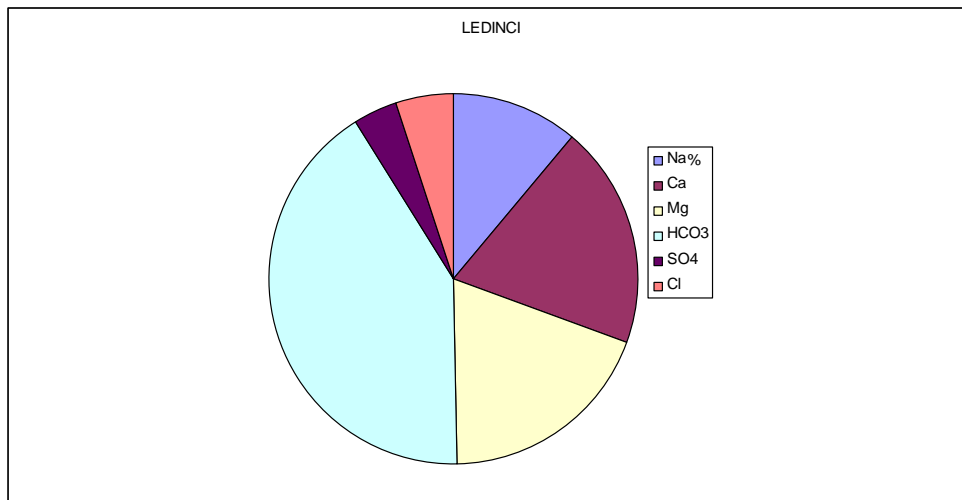


Figure 2. Pie chart of the chemical composition of the water content from the spring in the Old Ledinici

Overall, the physico-chemical parameters were not stable, which was expected from the aquifers having a free level and being fully open toward the surface field. Namely, in the last analysis made, the content of sulfate and chloride increased.

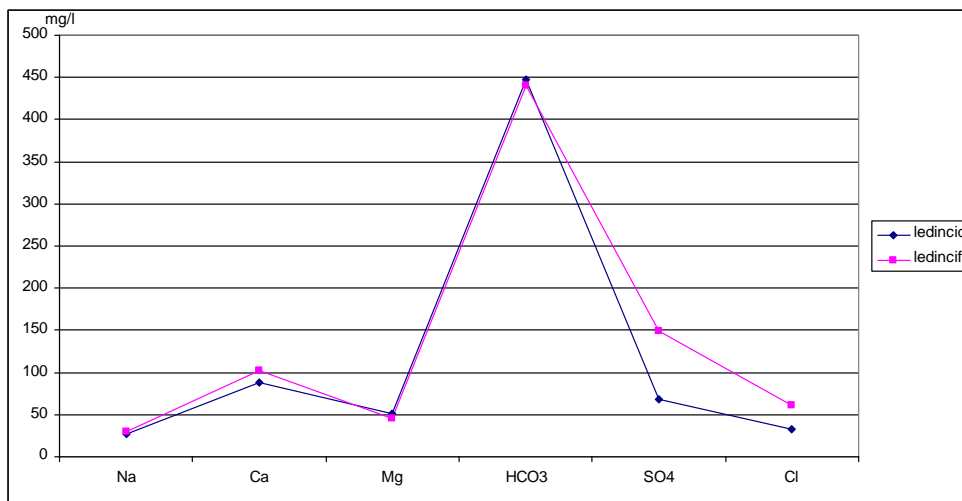


Figure 3. Sheler's permanence diagram of the major ions content in the spring water in the Old Ledinici

Of the micro components contained in the water, none was found to have increased its content. The manganese content was not recorded while the total iron content was below the maximum allowable limit. The content of organic matter in the water was expressed through the consumption of potassium permanganate ( $\text{KMnO}_4$ ), not exceeding the MAC drinking water, being higher over the dry season in December 2011 but lower in the wet February of 2012.

The content of ammonium ions did not exceed the MAC drinking water. The water contained more orthophosphates, boron, phenols than allowed by the regulations for hygienic quality of drinking water ("Off. Gazette of FRY", no. 42/98). Microbiological testing of the water was not done. It was not recommended for use till the entire procedure of the water facility sanitation was used and microbiological quality of water checked.

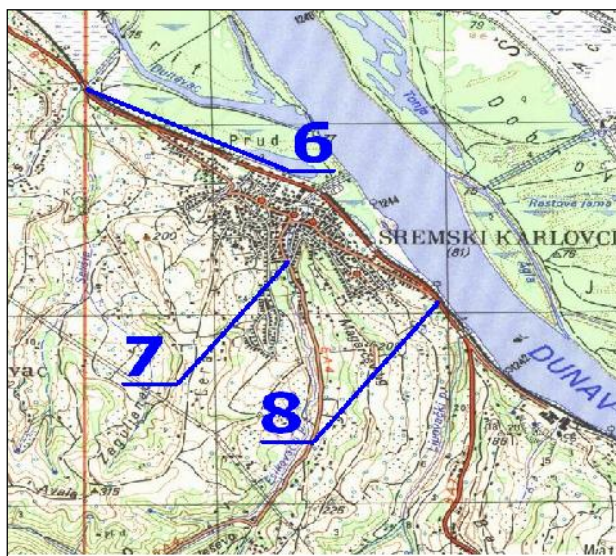


Figure 4. Geographical position of the spring : 6 -"Varadinska fountain", 7 – Stražilovski stream, 8 – the spring "Mutica"

The spring of cadastral number 6 in Sremski Karlovci "Varadinska fountain": is located on the right side of the road Novi Sad-Belgrade. The spring drains the paleo-landslide and is known from the Turkish period. This fountain had an icon of St. Elias and Archdeacon Stevan in the wall. Directly above the intake structure there is also a residential building with outbuildings. The trap is completely open and therefore easy to be polluted by surface water.

**Physical properties:** Abundance of the spring was constant 0.476 l/s, the water was clear, or almost clear. Water was odourless, water temperature 12.6-13.4°C. The pH of the test water index was 6.92-7.43, showing a neutral to alkaline medium. The values of the hardness ranged from 19-24.37°dH. According to Klute- this classification of water indicated that it belonged to the "hard" water, with mineralization being determined through the dry residue value obtained analytically and showing noticeable variations in the samples studied. The values of dry residues ranged from 345-1148 mg/l, averaging 530 mg/l. Conductivity was high, being within the range of 557-1851 S/cm, the average being of about 800 S/cm, visibly exceeding the MAC for drinking water.

**Chemical composition:** The investigation of the chemical composition of groundwater in the site denoted to the water analyzed to fall under hydrocarbon-class calcium - magnesium-sodium groups (according to the classification Alekin-a). The cation composition of calcium ions ranged from 70.7-102.4 mg/l, accounting for 44% eq. on average. The content of magnesium ions was found to range from 42-44 mg/l being 34.6% eq. The content of sodium varied from 21.3-28.1 mg/l, accounting for 21.1% eq. on average (Fig. 5, 6). The anion content was absolutely dominated by carbonate ions content ranging from 524.9-536.8 mg/l or 85.8% eq. on average. The total content of sulfate ions was found to vary from 13.3- 74.5% of 9.2 eq. while chlorides were reported to range from 7.4-27 mg/l, or 4.9% eq. on average. During the monitoring from 2003–2011, the variations of chlorides and sulfate were found to be high.

Except for boron, of all other micro-components contained in water, none had an increase in content. Manganese and total iron content were reported to range below the maximally allowable amount.

Content of organic matter in the water expressed through consumption of potassium permanganate (KMnO<sub>4</sub>) did not exceed the MAC potable water, but it did in the dry season in December 2011, but also it was lower in the wet in February 2012. Water contained

orthophosphates and phenols over allowable standards based on the regulations for hygienic drinking water ("Off. Gazette of FRY", no. 42/98).

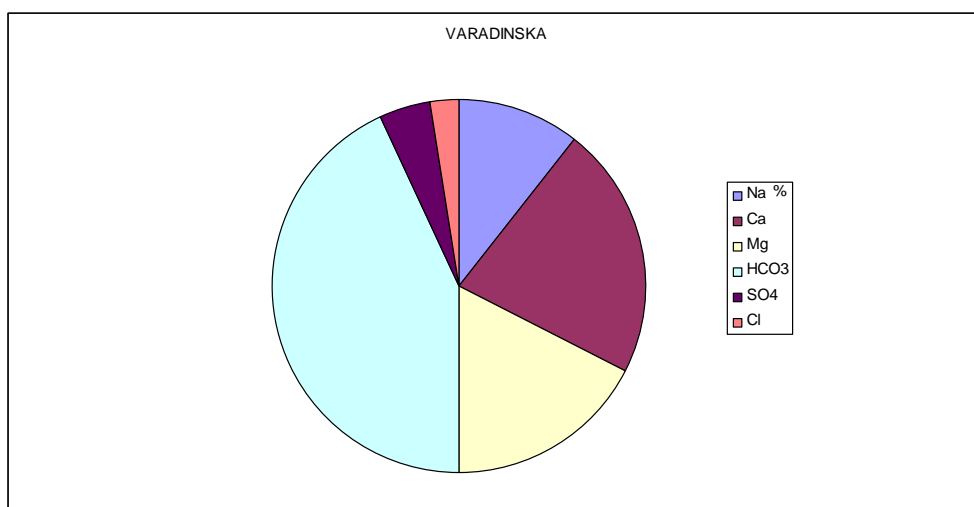


Figure 5. Pie diagram of the chemical composition of the spring water „Varadinska fountain“in Sremski Karlovci

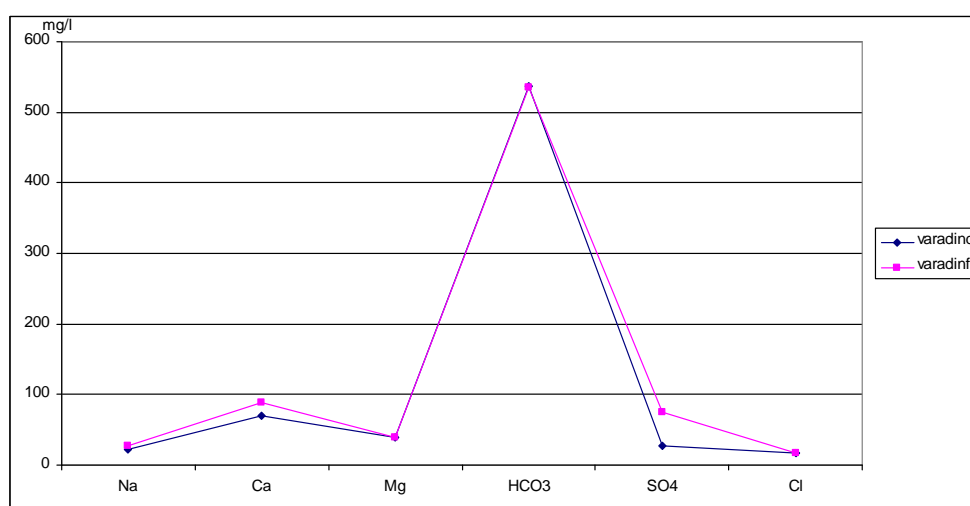


Figure 6. Sheler's diagram of the spring water major ions contained in the „Varadinska fountain“u Sremski Karlovci

Microbiological testing of water – the most frequent causes of microbiological incorrectness of water were established in 41 (61.19%) control samples of drinking water from public wells "Varadinska Fountain" for the period 2005-2011, with the findings of thermo-tolerant microorganisms in 38 (56.72%) controlled samples, followed by the increase in the total coliform microorganisms in 23 (34.33%) and controlled samples as well as in those of streptococci of fecal origin in 18 (26.87%) control samples.

The most commonly cultured microorganisms in drinking water from public well "Varadinska Fountain" in the period 2005-2011, appeared to be *Escherichia coli* (in 22 controlled patients, being 32.84% of the total number of microbiological analysis) and *Bacillus spp.* (in 20 samples of controlled, or 29.85% of the total number of microbiological analysis). It was not

recommended for use until the complete process of the facility water sanitation was applied and microbiological quality of water controlled.

The spring of cadastral number 8 in Sremski Karlovci "Mutica" located on the right side of the road Novi Sad-Belgrade, outside Sremski Karlovci. It drains moderate paleo-landslide. Directly above the intake structure there was a residential facility with the outbuildings. The cover of intake structure was in the level of the flat terrain and quite easy to go into the capping of surface water from higher elevation.

**Physical properties:** Abundance of sources varied from 0.32 to 0.27 l/s, the water was clear, or at the border, no smell. The water temperature was 11.4-13.6°C. The pH value of the index of the test water was 7.18 to 7.50, showing neutral to alkaline medium. The values of total hardness ranged from 23.9- 26.89°dH. According to Klute - this classification of water belongs to the "hard" water. Mineralization was found through the values of dry the residue obtained analytically appearing to be quite variable with the samples examined. The values of dry residues ranged from 494-657 mg/l, averaging 601 mg/l. Conductivity was high, ranging from 782-1081  $\mu\text{S}/\text{cm}$ , averaging about 900  $\mu\text{S}/\text{cm}$ . In the period of monitoring of the project it exceeded the MAC for drinking water.

**Chemical composition:** The investigation of the chemical composition of groundwater at the source denoted to the water analyzed to belong to hydrocarbon classes, magnesium-calcium-sodium group (according to the classification Alekin-a). The cation composition of calcium ions ranged from 79-102.1 mg/l prevailing by 39.3% eq. on average. The content of magnesium ions was found to range from 53-58.5 mg/l or 39.7% eq., whereas the content of sodium varied from 22.5-28.1 mg/l or 20.8% eq. on average (Fig. 7, 8). As for the anion content, carbonate ions were absolutely dominant, with their content ranging from 523.4-553.9 mg/l or 85.6% eq. on average. The total content of sulfate ions varies from 40.8-93.8% amounting to 6.5 eq. while chlorides were found to range from 1.8-28.7 mg/l or 8% eq. average. During the monitoring from 2003–2011, the variations in chlorides and sulfates were noticeably high.

Throughout the final analysis made, sulfate and chloride content were revealed to be higher. Of the micro components in water, no increase in content of any element other than boron was revealed. Occasionally, as a result of pollution, cadmium, nitrogen triad, potassium and arsenic appeared in unallowable concentrations. The content of organic matter in the water expressed through the consumption of potassium permanganate ( $\text{KMnO}_4$ ) did not exceed the MAC in drinking water, except for water sampled in February 2012. The water of each sample contained orthophosphates and phenols over allowable limit confirmed by regulations for hygienic standards of drinking water ("Off. Gazette of FRY", no. 42/98).

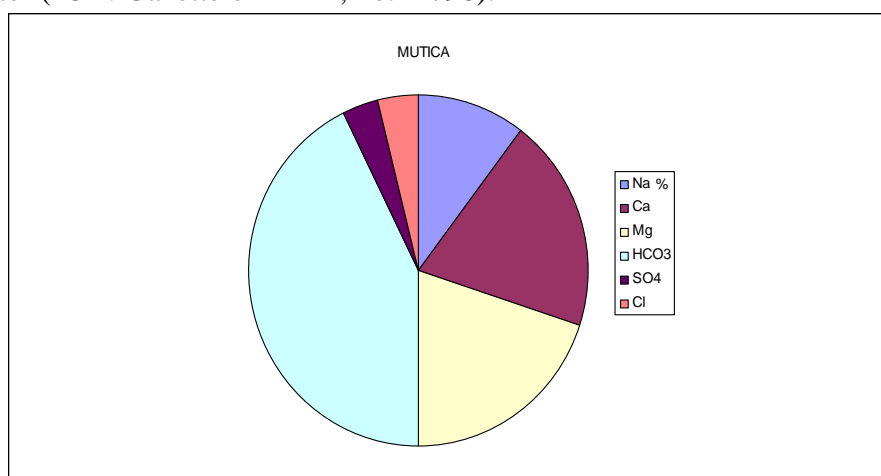


Figure 7. Pie diagram of the chemical composition of the spring water „Varadinska fountain“in Sremski Karlovci

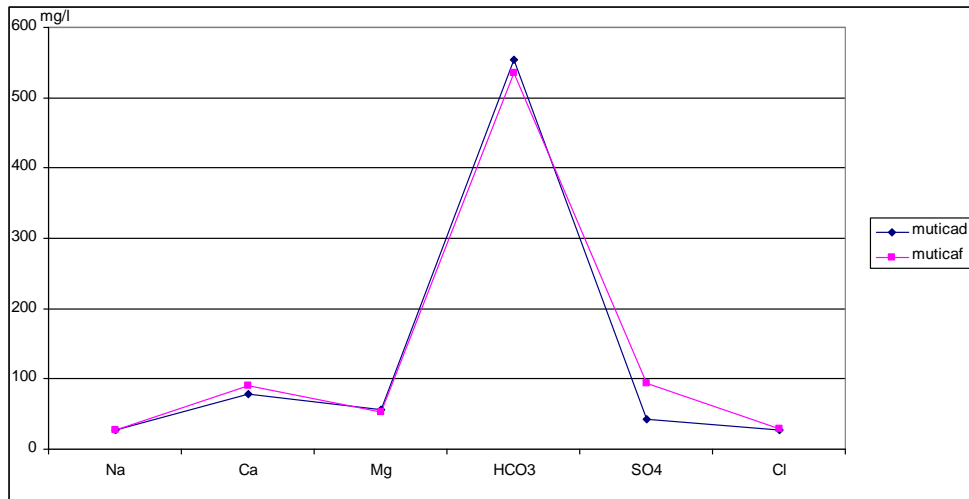


Figure 8. Sheler's diagram of the spring water major ions content of the „Varadinska fountain“ u Sremski Karlovci

Tabela 1. Results of physico-chemical analyses made on the spring waters in Old Ledinci i Sremski Karlovci

Parameters ( mg/l)	Ledinci (5)	Sample (5)	Petro. esma (6)	Sample (6)	Mutica (8)	Sample (8)	MAC drinking water
	22.12.2011.	23.02.2012.	22.12.2011.	23.02.2012.	22.12.2011.	23.02.2012.	
<b>EC(μS/cm)</b>	<b>1061</b>	<b>1108</b>	936	917	<b>1081</b>	<b>1077</b>	<b>1000</b>
<b>NH<sub>4</sub></b>	0	0.07	0	0.06	0	0.07	<b>0.1</b>
<b>Nitrates</b>	8.07	12.8	10.39	13.25	25.04	21.83	<b>50</b>
<b>Nitrites</b>	0	0	0	0	0	0	<b>0.03</b>
<b>KMnO<sub>4</sub></b>	4.9	2.3	2.6	0.73	2.6	<b>15.2</b>	<b>8</b>
<b>HPK</b>	2.75	0.58	2.75	0.18	2.75	3.8	-
<b>SO</b>	683.5	699.4	533.3	510.7	657.5	628.8	-
<b>Chlorides</b>	32.3	60.9	17.4	16.7	27.7	28.7	<b>200</b>
<b>Bicarbonates</b>	447.7	441	536.8	534.9	553.9	534.9	-
<b>Sulfates</b>	68.1	149.7	27	74.5	43.2	93.8	<b>250</b>
<b>Total iron</b>	0.08	0.04	0.04	0.03	0.03	0.05	<b>0.3</b>
<b>Calcium</b>	87.5	102.9	70.7	88.5	79	90	<b>200</b>
<b>Magnesium</b>	51.5	45.5	40	40	<b>56</b>	<b>53</b>	<b>50</b>
<b>Sodium</b>	27	29.4	22.2	28.1	28.1	27	<b>150</b>
<b>Potassium</b>	4.4	3.6	4.3	3.5	<b>12.7</b>	12	<b>12</b>
<b>pH</b>	7.4	7.2	7.1	7.1	7.3	7.2	<b>6.8-8.5</b>
<b>Ortophosphates</b>	<b>0.39</b>	<b>0.5</b>	<b>0.27</b>	<b>0.38</b>	<b>0.36</b>	<b>0.4</b>	<b>0.15</b>
<b>Phenols</b>	<b>0.003</b>	<b>0.079</b>	<b>0.004</b>	<b>0.118</b>	<b>0.008</b>		<b>0.001</b>
<b>Ba</b>	0.47	0.32	0.6	0.32	0.6	0.33	<b>0.7</b>
<b>Total Cr</b>	0.03	0.02	0.03	0.02	0.03	0.03	<b>0.05</b>
<b>Pb</b>		0.01		0.01		0.01	<b>0.01</b>
<b>Boron</b>	<b>0.05</b>	<b>&lt;0,5</b>	<b>0.01</b>	<b>&lt;0,5</b>	<b>0.01</b>	<b>&lt;0,5</b>	<b>0.3</b>

\* the values marked with red colour are above the MAC for drinking water.

### Conclusion

Spring waters are of low mineralization. According to hardness, they belong to the category of "hard" waters. By classification of Alekin – the waters belong to hydro-carbonate class of water, of calcium-magnesium-sodium type. Overall, physical-chemical parameters were not stable as being expected from aquifers having a free level, as being entities open toward the soil surface, also with the trap in these wells being lower than the ground level, so that the surface water along with seasonally scattered pollutants from the surface flow into the catchment area.

Based on the analysed chemical parameters, the water did not meet the requirements of the drinking water because of physical-chemical improperness: due to increases in concentrations of orthophosphates, phenols and boron in all the samples analysed, further, due to occasional organoleptic changes in the water examined and due to its constant bacteriological improperness.

Therefore, it is not recommended to use as drinking water without water treatment being completely performed.

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## EFFECT OF HEAVY METALS ON THE MICROBIAL ACTIVITY OF SOILS UNDER RED CLOVER

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### Abstract

Heavy metals reach the environment, primarily soils, mostly through human activities. They enter certain biological links in nutrient and energy cycles, thus becoming a source of many environmental problems and health issues in both people and animals. Microorganisms as an important link in the cycling process can serve as indicators of both soil pollution and potential toxicity to other biological systems.

The objective of this study was to evaluate the effect of different concentrations of lead (60; 120; 250 mg kg<sup>-1</sup> soil) and mercury (1,0; 2,0; 4,0 mg kg<sup>-1</sup> soil) on total microbial count and Azotobacter in two soils, a Vertisol and an alluvium under red clover cultivation in three growing seasons. The experiment was conducted under greenhouse conditions at the Faculty of Agronomy, a ak.

Numbers of the microorganisms tested were determined by indirect counting methods involving plating out a soil suspension onto appropriate selective culture media.

Depending on the type and concentration involved, heavy metals had a significant effect on soil microbial count in the alluvium and vertisol during the red clover growing season. Low concentrations of lead and mercury (60 mg kg<sup>-1</sup> and 1 kg<sup>-1</sup> soil, respectively) did not lead to significant changes in total microbial and Azotobacter counts. At 250 mg kg<sup>-1</sup> soil, lead induced a decrease in total microbial and Azotobacter counts. Mercury had a markedly higher depressive effect on soil microorganisms, with concentrations of 2 and 4 mg kg<sup>-1</sup> soil significantly reducing the total microbial and Azotobacter counts.

**Keywords:** heavy metals, microorganisms, plant, soil.

### Introduction

Soil is among major natural resources invaluable to mankind as a whole, rather than to a single generation, nation, group of people or an individual. Soil formation is a slow process compared with the rapid rate of soil degradation resulting primarily from uncontrolled human-related activities. In this regard, heavy metals have received particular attention as they are very difficult to degrade, in contrast to organic pollutants (Kabata-Pendias, 2001), and show a tendency to enter the biological nutrient cycle, thus causing many environmental problems and health issues (Cornelija and Franc, 2004; Majer et al., 2002). Soil remediation must include stabilisation techniques that are typically short-term and costly (Brovn, 1997). Therefore, the central tendency of modern integrated primary production of food is to reduce and control the use of agrochemicals and other types of indirect soil pollution. A variety of methods are used to indicate the presence of heavy metals in the soil. Recent research has justified the use of microorganisms as the most active elements of the soil to this end (Rundella et al., 2005; Rajapaksha, 2004). The effects of heavy metals on soil microorganisms are dependent on their type and concentration, soil physicochemical characteristics, plant cover, cultural operations, etc. Some of them (cobalt, chromium, nickel, iron, zinc, etc.), when found at low concentrations, play an important role in plant and microbial nutrition, whereas some others, even at low concentrations, produce adverse effects on the biogeosphere (Bruins et al., 2000). High

concentrations of either group are toxic to soil microorganisms (Lee et al., 2002). Toxicity is generally exhibited through their adsorption to the cell surface, resulting in changes in cytoplasmic membrane permeability, cytoplasmic protein coagulation, enzyme inactivation, DNA damage, etc. (Sobolev and Begonia, 2008). This indirectly leads to changes in the structure of microbial cenoses in the soil as well as to the emergence of heavy metal tolerant microorganisms (Spain and Alm, 2003) or toxinogenic microorganisms, which indirectly induces soil pollution.

The objective of this study was to evaluate the effect of different concentrations of lead (60; 120; 250 mg kg<sup>-1</sup> soil) and mercury (1,0; 2,0; 4,0 mg kg<sup>-1</sup> soil) on the total microbial and *Azotobacter* counts in a vertisol and an alluvial soil under red clover.

### Material and methods

The experiment was established under controlled greenhouse conditions at the Faculty of Agronomy, a ak. The test plant was red clover cv. “Kruševa ka K32” sown on two soil types (factor B) viz. a degraded alluvium (pH<sub>KCl</sub>- 6.4; humus-1.7%; N-0.1%; P<sub>2</sub>O<sub>5</sub>-0.068 mg g<sup>-1</sup>; K<sub>2</sub>O- 0.1 mg g<sup>-1</sup>) and a vertisol (pH<sub>KCl</sub>-5.01; humus- 3.59%; N-0.20%, P<sub>2</sub>O<sub>5</sub>-0.029 mg g<sup>-1</sup>; K<sub>2</sub>O-0.26 mg g<sup>-1</sup>) in 15 dm<sup>3</sup> containers in five replications, according to a randomised block design. Soil samples were collected from areas confirmed to be unpolluted by laboratory analysis. Red clover was planted at a spacing of 5-7 cm and a depth of 1cm. Upon planting, the soil was subjected to experimental treatments involving the addition of an aqueous solution of heavy metals (factor A): lead [as Pb(NO<sub>3</sub>)<sub>2</sub>] at 60; 120; 250 mg kg<sup>-1</sup> soil and mercury (as HgCl<sub>2</sub>) at 1.0; 2.0; 4.0 mg kg<sup>-1</sup> soil. Treatments without the addition of heavy metals were used as the control.

The soil was sampled for microbial analysis using a laboratory probe three times during the red clover growing season (factor C): I- at the 2-3 leaf stage; II- at the intensive plant growth stage; III- at budding. Microbial analysis involved determination of the total microbial and *Azotobacter* counts by indirect plating on a soil agar (Pochon and Tardieux, 1962) and Fyodorov's medium (Anderson, 1958), respectively. Results were subjected to a three-factor analysis of variance, and the significance of differences was assessed by the LSD test (Statistica SPSS 5).

### Results and discussion

The results of the present study show the dependence of the effect of heavy metals on their type and concentration, sampling date and soil type (Tabs. 1-4).

Overall, the low levels of lead (60 and 120 mg kg<sup>-1</sup> soil) did not have a significant effect on the total microbial count. However, they exhibited selective effects, depending on soil type and red clover growing stage (Tab. 1). Namely, an initial depressive effect of lead at a concentration of 120 mg kg<sup>-1</sup> was observed only on the alluvial soil, as opposed to the slight stimulating effect on the vertisol. During the growing season, the differences levelled off on the alluvium as well, reaching the level observed in the control treatment. The lead concentration of 250 mg kg<sup>-1</sup> soil (2.5-fold higher than the permissible levels prescribed by the European Council Regulation 2092/91) led to a significant decrease in the total microbial count, particularly in the alluvium (Tab. 1). The slight decline in microbial numbers in the vertisol was likely due to its somewhat more favourable physicochemical properties (primarily the organic component or humus content). This suggestion is supported by the results of Bais et al. (2006) and Alidoust et al. (2012), who underlined the importance of organic matter and plant exudates in reducing the mobility of some heavy metals in the soil, and their availability to both plants and other biological components in the soil. The lower effect of heavy metals at final growth stages of red clover may be associated with the cometabolic effect of organic root exudates and the ability for adaptation and partial detoxication of toxinogenic components by other soil microorganisms (Khan and Scullion, 2002, Rajapaksha et al., 2004).

Tab. 1. Effect of lead (Pb<sub>1</sub> – 60.0; Pb<sub>2</sub> – 120.0; Pb<sub>3</sub> - 250 mg kg<sup>-1</sup> soil) on total microbial count (10<sup>6</sup> g<sup>-1</sup> absolutely dry soil) in the alluvium and vertisol during red clover growing season

A		Control		Pb <sub>1</sub>		Pb <sub>2</sub>		Pb <sub>3</sub>		$\bar{X}$ C	
B		Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol		
C	I	88	75	90	82	71	77	70	60	76.62	
	II	98	77	101	79	93	80	75	73	84.50	
	III	140	96	136	101	137	102	131	103	118.75	
$\bar{X}$		95.66		98.16		93.33		85.33			
$\bar{X}$		Alluvium	102.50								
		Vertisol	83.75								
Lsd		A	B	C	AB	AC	BC	ABC			
0.05		4.64	2.50	3.00	6.54	8.04	5.69	11.32			
0.01		6.12	3.30	3.98	8.64	10.64	7.53	14.98			

The application of 60 and 120 mg Pb kg<sup>-1</sup> soil did not lead to significant changes in Azotobacter count throughout the red clover growing season (Tab. 2). This may be attributed to the morphophysiological characteristics of these microorganisms (they produce a mucilaginous sheath on the exterior of the cell that ensures complexation and inactivation of different toxicants, and heavy metals as well), as evidenced by the research conducted by Govedarica et al. (1993). However, the high concentration of this metal (250 mg kg<sup>-1</sup> soil) significantly reduced the count of these microorganisms, particularly at the first two experimental stages in the alluvium.

Tab. 2. Effect of lead (Pb<sub>1</sub> – 60.0; Pb<sub>2</sub> – 120.0; Pb<sub>3</sub> - 250 mg kg<sup>-1</sup> soil) on Azotobacter count (10<sup>2</sup> g<sup>-1</sup> absolutely dry soil) in the alluvium and vertisol during the red clover growing season

A		Control		Pb <sub>1</sub>		Pb <sub>2</sub>		Pb <sub>3</sub>		$\bar{X}$ C	
B		Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol		
C	I	27	14	30	16	26	13	18	10	19.25	
	II	29	16	27	20	31	15	27	13	22.25	
	III	41	22	44	21	39	24	33	17	30.12	
X		24.83		26.33		24.66		19.66			
X		Alluvium	31.00								
		Vertisol	16.76								
Lsd		A	B	C	AB	AC	BC	ABC			
0.05		2.55	1.38	1.65	3.59	4.42	3.13	6.23			
0.01		3.37	1.18	2.19	4.75	5.85	4.14	8.24			

Mercury was found to be extremely toxic to the soil microflora; therefore, the reduction in total microbial count was also observed in treatments with 2.0 mg Hg kg<sup>-1</sup> soil. An even more depressive effect of mercury was induced by the highest application rate (4.0 mg kg<sup>-1</sup> sol), particularly in the alluvial soil (Tab. 3).

Tab. 3. Effect of mercury ( $Hg_1 - 1.0$ ;  $Hg_2 - 2.0$ ;  $Hg_3 - 4.0$  mg  $kg^{-1}$  soil) on the total microbial count ( $10^6$   $g^{-1}$  absolutely dry soil) in the alluvium and vertisol during the red clover growing season

A	Control		$Hg_1$		$Hg_2$		$Hg_3$		$\bar{X}C$	
B	Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol		
C	I	88	75	89	70	68	63	57	54	70.50
	II	98	77	96	81	69	70	60	64	76.88
	III	140	96	142	105	113	75	69	76	102.00
$\bar{X}$	95.66		97.16		76.33		63.33			
$\bar{X}$	Alluvium	90.75								
	Vertisol	75.50								
Lsd	A	B	C	AB	AC	BC	ABC			
0.05	5.82	3.15	3.81	8.25	10.08	7.10	14.31			
0.01	7.68	5.17	5.04	10.92	13.35	9.42	18.93			

Azotobacter was found to be highly susceptible to mercury, with its counts decreasing by about 39 and 54% in treatments with low ( $2.0$  mg  $kg^{-1}$  soil) and high ( $4.0$  mg  $kg^{-1}$  soil) mercury application rates, respectively, compared to the control (Tab. 4). As in the case with the soil microflora, the depressive effect of mercury on Azotobacter counts was also particularly remarkable during the early stages of the growing season and in the alluvial soil. The markedly toxic effect of mercury on most soil microorganisms was also reported by other authors (Casucci et al., 2003; Mandic et al., 2010).

Tab. 4. Effect of mercury ( $Hg_1 - 1.0$ ;  $Hg_2 - 2.0$ ;  $Hg_3 - 4.0$  mg  $kg^{-1}$  soil) on Azotobacter counts ( $10^2$   $g^{-1}$  absolutely dry soil) in the alluvium and vertisol during the red clover growing season

A	Control		$Hg_1$		$Hg_2$		$Hg_3$		$\bar{X}C$	
B	Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol		
C	I	27	14	23	17	13	8	8	7	14.63
	II	29	16	27	14	19	13	13	8	17.38
	III	41	22	39	25	21	17	22	11	24.75
$\bar{X}$	24.83		24.16		15.16		11.50			
$\bar{X}$	Alluvium	23.50								
	Vertisol	14.33								
Lsd	A	B	C	AB	AC	BC	ABC			
0.05	2.49	1.33	1.63	3.51	4.29	3.06	6.07			
0.01	3.28	1.76	2.16	4.64	5.68	4.06	8.04			

Overall, the counts of the microorganisms tested were considerably higher in the alluvium than in the vertisol, and increased during the red clover growing season.

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## Conclusion

Depending on the type and concentration involved, heavy metals had a significant effect on soil microbial count in the alluvium and vertisol during the red clover growing season. Low concentrations of lead and mercury ( $60 \text{ mg kg}^{-1}$  and  $1 \text{ kg}^{-1}$  soil, respectively) did not lead to significant changes in total microbial and Azotobacter counts. The high application rate of lead ( $120 \text{ kg}^{-1}$  soil) had a depressive effect on the microorganisms tested only at the early stages of the growing season, particularly in the alluvial soil. At  $250 \text{ mg kg}^{-1}$  soil, lead induced a decrease in total microbial and Azotobacter counts. Mercury had a markedly higher depressive effect on soil microorganisms, with concentrations of 2 and  $4 \text{ mg kg}^{-1}$  soil significantly reducing the total microbial and Azotobacter counts.

Overall, the counts of the microorganisms tested were considerably higher in the alluvium than in the vertisol, and increased during the red clover growing season.

The present findings suggest that soil microorganisms can serve as an indicator of heavy metal pollution in soils.

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## ANALYSIS OF REVENUE STRUCTURE IN FINANCING NATIONAL PARKS IN SERBIA

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### Abstract

Financing of protected areas in Serbia is one of the important issues in the system of nature protection. Protected areas covers ~5,89 % of territory of Serbia while national parks involve ~30,57% of this land. Their importance is very significant since they include nature ecosystems, landscape and cultural features and this area is intended for protection of existing natural resources as well as for protection of landscapes, geological and biological features. Their usages are also oriented toward scientific, educational, cultural and tourism needs. This paper analyses different revenue structures in managing this type of natural resource as well as approaches in the financing in the region and abroad. The objective of the article is the structure of revenues in the financing five national parks in Serbia. Territorial framework of the research is the Republic of Serbia with the autonomous provinces. In order to determine the structure of revenues in the financing, statistical techniques based on analysis of time series is used. Non-reactive method is used for collection of data's. Results are showing that most revenues are coming from sales and services and least from government revenue allocation. The best average exponential trend has national park "Fruška gora" while national park "erdap" has negative exponential trend. Based on this, in all national parks, on revenue structure mostly influence revenues from sales of goods and fees.

**Key words:** nature protection, national park, revenue structure, financing, trends

### Introduction

Serbia as a future member of the European Union (EU) has already begun the process of harmonization of its legislation and adoption of certain norms and rules in the management of protected areas (PA). System of protected areas in Serbia is a complex structure of different actors, rules, responsibilities and institutions involved in the management of PA. Law on nature protection (2009) is one of the essential documents of nature protection in Serbia. This law defines the different forms of management, types and methods of funding the PA. This law also defines institutions involved in the management of the national park. Protected areas are defined as areas that have a specific geological, biological, ecosystem and/or landscape diversity and therefore, based on act on the protection, are proclaimed as protected areas of common interest. On the other hand, the International Union for Conservation of Nature (IUCN) defines a protected area as a geographical space, recognized, marked and managed through the implementation of legislative and other effective measures<sup>19</sup>, in order to achieve long-term protection with associated "ecosystem services" and cultural values (Emerton et al., 2006). The national park (NP) represent "... an area with number of different natural ecosystems of national significance, the prominent feature of the natural landscape and cultural heritage in which man lives in line with the nature, intended for the preservation of existing natural values and resources ....."(2009). Management of NP in Serbia is given to public enterprises (PE) registered for this activity. Thus, in Serbia, there are five public enterprises responsible for the management of this good (Table 1)

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<sup>19</sup> This means that PA must be recognized through specific laws, international conventions and agreements and manage effectively in accordance with legislation and traditional usage.

Table 1. National parks in the Republic of Serbia

National park	Area (ha)	Manager– Public enterprise national park (PENP)	Region of Serbia	Year of establishment	Year of establishment of current status	IUCN category
Fruška Gora	25393	PENP "Fruška gora"	AP Vojvodina	1960	1993	V
erdap	63608,45	PENP "erdap"	Central Serbia	1974	1993	IV
Tara	19175	PENP "Tara"	Central Serbia	1981	1993	II
Kopaonik	11809,91	PENP "Kopaonik"	Central Serbia	1981	1993	V
Šar Planina	39000	PENP "Šar planina"	AP Kosovo i Metohija	1986	1993	II
Total area	158986,4					

Source: Sekulic, 2011 and original

Financing of NP is directly defined by the Law on nature protection and the Law on forests, and indirectly through a number of by-laws and regulations. In the system of financing NP three types of funding are defined (2009): a) budget of the Republic of Serbia-budget grants, b) revenue generated by the organization that manages the NP and c) donations. Direct funding through budget grants may be from the budget of Republic of Serbia, autonomous province (AP) and the funds of local governments. Revenues generated by the organization that manage NP may be fees for uses of PAs, income generated through performing activities and management of NP and funds obtained from implementation of programs, plans and projects in the field of environmental protection. The last category includes donations, gifts and grants, from domestic and international source.

In order to analyze the revenue structure in the financing of the national parks, public enterprise, as a form of business, needs to be defined. Enterprise represents the main organizational form in the process of reproduction, which function is related to the satisfaction of social needs for specific products and/or services (Rankovic, 2008) and represents legal entity which carries on business for profit. Owners of enterprises may be legal, individuals, state or local government. Public enterprise perform duties of special and general interest (Zivkovic, 2006), and in its management is state involved, through its representatives. Public enterprises are established by the state, autonomous provinces and local self-governments. They are formed to perform tasks that are in the area of infrastructure, public services, important government economic systems and the exploitation of natural resources that are of public interest. Revenue structure of NP, which is the subject of this research (Table 2), in this paper, is shown through different revenue categories which are involved in the financing of NP. Revenues involved in the financing of NP are showed in Table 2 (Zivkovic, 2006 Rankovic, 2008).



Table 2. Type of revenues in the management of national parks

Type of revenue	Distribution of revenue type
Operating revenue (operating income represents income that companies obtain from its operations, i.e. their core business)	Revenues from sales of goods and services
	Revenues from donations
	Fee revenues
	Other operating revenues
Revenue from capital	Interests, share of profit in other corporations, positive difference between selling price and nominal value of shares sold, renting of fixed assets etc. (Rankovic, 2008)
Extraordinary revenue	Result of extraordinary and unforeseen circumstances. These revenues are coming from charges of previous bad debts, obligations subscribed, positively resolved law suit, etc. (Rankovic, 2008)

Source: Živkovic, 2006, Rankovic, 2008

The research topic of this study is revenue structure in the financing of NP in Serbia. The main objective is to determine the revenue structure and its trend, and determine cause-effect relationship between observed values. The purpose of the research is that by comparing the revenue structure in five national parks, define necessary facts that will enable finding the most appropriate option for financing these categories of PA in Serbia.

Based on this two hypothesis are set:

- there is growing impact of revenues from goods and services on overall revenues, based on total revenues per hectare of protected area;
- average growth rate of fees and budget grants is on the level of growth rate of all operating revenues.

### Material and method

The research is conducted within the NP's in order to determine the structure of revenues and revenues structure trends of the national parks. As a research method in this paper, method of trend analysis (Šešić, 1984) is used. In order to determine structure of revenues in the financing of the national parks statistical techniques based on the analysis of time series are used (Ke a et al., 2012, Stojkovic, 2001). As a basic-parameters following parameters are used:

- ) absolute level of occurrence;
- ) mean absolute level of occurrence;
- ) average annual exponential growth rate (*I<sub>s</sub>*).

As a special scientific method, for the collection of financial indicators in the Republic of Serbia, non-reactive method is used (Neuman, 2006). This method involves research that does not involve the collection of data directly from the subject of research and is contrary to the research methods such as interviews, surveys and experiments. The basic techniques of non-reactive research methods include content analysis, use of existing statistics, documents and their secondary analysis.

### Results and discussion

The results are showing that financing of NP is primary affected by sales of goods and services as well as income from fees (Table 3). National park erdap (NP ) from all observed NP only has negative *I<sub>s</sub>* (-0.4%). This value is mostly affected by strong negative *I<sub>s</sub>* from sales (-35.1%) and fee revenues (-6.3 %). On negative *I<sub>s</sub>* from revenue in NP mostly influence results from last year (2011), which compared to the highest income in this category represent a reduction of almost 80%.

Other NP recorded positive *I*s and among them stands out NP Šar planina (NPŠP), which in 2009 and 2010 recorded significantly higher sales, but far lower incomes ( $10\text{-}228 \text{ RSD}\cdot\text{ha}^{-1}$ ) compared to the other NP. Differences between revenue sales are primarily present between NP and other national parks. The reasons for this are primarily affected by large share of non-productive protective forest (bushes and shrubs) in the territory NP, which are not affordable to use. However, from the other side, NP Tara (NPT) and the National Park Kopaonik (NPK), recorded lower revenues from NP Fruška Gora (NPF), although the condition of forests is far more favorable in this two NP. NPT and NPK have large share of high-quality forest (dedicated unit 10) with a favorable assortment structure and high share of technical wood. On the other hand, NPF is mostly covered with soft hardwoods, which has far lower assortment structure (from two other mentioned NP), but are much easier to sell as technical wood (technical logs) because of so-called positional rent. In Montenegro revenues from selling forest assortments are negligible. The reason for this lays in strict protection of valuable forests which doesn't leave space for forest management activities. Influence on this has also structure of the forest complex that is much different from the theoretical normal (Curović et al., 2013).

Table 3. Annual exponential revenues growth rate of national parks in Serbia in period from 2008-2011.

National park	Overall business revenues (%)	Revenues from sales and goods (%)	Revenues from budget grants (%)	Revenues from fees (%)	Other business revenues (%)	Extraordinary revenues (%)	Financial revenues (%)	Overall revenues (%)
erdap	-2,3	-35,1	9,5	-6,3	24,5	30,1	79,2	-0,4
Fruška gora	11,4	8,1	-21,8	20,5	175,4	5,4	-19,1	10,7
Kopaonik	5,0	8,0	-2,9	8,0	/*	-26,5	9,4	1,9
Tara	4,0	4,9	14,0	11,9	27,9	2,7	-19,1	3,9
Šar planina	21,2	82,4	13,6	/*	/*	58,7	/*	14,2
Prose na Is	<b>7,1</b>	6,3	<b>1,4</b>	<b>11,0</b>	<b>18,5</b>	<b>2,6</b>	<b>18,1</b>	<b>5,8</b>

\* due to the lack of data in some years of observation in this category of *I*s isn't possible to calculate

**Chart 1.** Income share in all national parks in Serbia in period between 2008-2011.

Source: original

Revenue from grants, compared to other income categories (Figure 1), recorded the lowest *Is* (1.43%). The reason for this lies in the negative *Is* in two NPs. The NPFG negative *Is* is very present (-21.8%) while NPK has somewhat mild character (-2.9%). Although revenues from grants have small *Is*, their impact on total revenues of NP is notable, considering the average share of income of 6.7%. Allocation of budget grants in NP, from budget of Republic of Serbia, has negative *Is* (-0.8%) and in average only 0.14% of the state budget. In Croatia NP funding is provided through the responsible Ministry of Culture and it amounts to 31% of all revenues. Comparing to other protected areas in Croatia (nature park, nature reserve and PA at the level of local municipalities) funding by the relevant ministry is about 90% of all revenues (Spurgeon, 2009).

**Chart 2.** Budget grant share of RS (%)

**Chart 3.** Share of GDP in financing NP

Source: original

Source: original

Allocations of budget grants in the Republic of Serbia in the 2008th year, in relation to gross domestic product (GDP), is 0.0025%, while spending in the Republic of Croatia in the same period for NP is 0.0052% (2008 and Spurgeon, 2009). Here is also present negative *Is* (-2.6%). For all protected areas in Croatia during 2004 state allocated 0.028% of GDP (Mansourian et al., 2008) while for Serbia, such data isn't available especially for longer period.

On the other hand, revenues from fees accounted approximately 13.8% of total revenues, and their *Is* has positive trend (11.0%). The results are showing that NP only has a negative *Is* since this revenue category earned twice less in period between 2009-2010. Revenues achieved in this category for NPSP are insignificant in comparison with the other NP. The reason for this surely lies

in the political situation that is present in the territory of Kosovo and Metohija, which prevents the collection of revenues from this category. The highest *Is* is present in NPPFG (20.5%), which in absolute terms, is double (for NP ) and quadruple (for NPT). The reasons for this are probably similar to the trend of revenues of goods and services. Road fees for the management of NP represent very efficient mechanism for financing PA. In Serbia, these fee goes directly to NP that manage the area, while in Croatia this fee is collected by the relevant ministries (Porej et al., 2009). In Spain, for example, the share of income (5-10%) of the highway that passes through the PA, are given to specific programs to protect species and habitats (Spurgeon, 2009). Fees collected, by providing touristic activity, in the world are a major source of income for PA. Thus, in studies that have been done in the 90's it was found that nearly half of all PAs charge entering in the PA (Giongo et al., 1994) and during the last decade this trend increased (Spurgeon, 2009). Although NPs in Serbia are one of the main destinations for sightseeing of PAs there is no record of this type of income. In Slovenia, the entry into the PA, is charged and makes 26% of all revenues, in Montenegro 7.5%, Greece 35%, Croatia 63% (Mansourian et al., 2008).

Other operating revenues, in all NP, follows *Is* of 18.5%, which in our case is the highest but it is influenced by *Is* that could not be calculated in the case of NPK and NPŠP. The average annual exponential growth rate of 175.4% in NPPFG is primarily influenced by the revenue from the 2008th which have been significantly reduced due to the effect of lowering the value of goods of about 21 million RSD. Extraordinary revenues, after income from grants, have the lowest average *Is* (2.7%). For this category of revenue, highest difference is reported in terms of total income (3-40%). So NP has the highest *Is* in this category and the largest growing share in total income (from 16% to 42%) while NPK recorded negative *Is* (-26.5%) and decreasing share in total income (from 16% to 6%). NPŠP has *Is* of 58.7% as a result of the large share of these revenues in 2009-2010. The average annual exponential growth rate for NPT is the lowest (2.72%) and the lowest recorded deviations (about 5% of the income), followed with *Is* of NPPFG (5.4%). High revenues of NP in this category are primarily the result of revenue valuation adjustments and suspension of the long-term reservation, which occurs as a result of unpaid claims and release of reserved funds for a particular purpose.

The last categories of revenues are from capital, which *Is* for NPs is at extremely high level (18.10%). This trend is right behind *Is* of other operating revenues and records high *Is* for NP (79.2%), negative *Is* for NPPFG (-19.1) and NPT (-19.1). On this trend mostly influence interest income, which for NP grows while for NPPFG and NPT decline. Although this revenue category has significant *Is*, they are negligible as a share of this value is less than 1% of total revenue. The exception here only makes NPŠP, which in 2011 records high revenues (16% of total revenue) arising due to repayment for pension and disability insurance.

### Conclusion

Based on the obtained results, it can be said that most revenues is earned through sale of goods and services (60.8%), followed by fee income (13.8%), other income (12.8%) and income from grants (6.7%). Looking at the structure of *Is* NPPFG has the best results. The average annual exponential growth rate of fees and revenues, from the sale of goods and services, have a positive impact on the total revenue of *Is* (10.7%), but this is influenced by other operating revenues, caused by the effect of lowering the value of the goods. NP recorded negative *Is* as a result of negative sales and fees (although this two NP have only negative operating revenue *Is*). NPT and NPK have similar overall *Is* that are results of positive *Is* of sales and fees, but because of the small share of overall revenues (although this two NP have negative *Is* in other categories), they do not reflect the whole picture. Looking at the *Is* of NPŠP, it could come to the conclusion that they have best position, but this can't be said with certainty because of very low revenues per hectare in various categories and the absence revenues from fees.

According to this, on the revenue structure, greatly affect revenues from sales of goods and services as well as revenues from fees. Other operating revenues together with financial revenues have the highest *Is* but their influence is much smaller due to the small share in total revenues. Interestingly, the high percentage of total revenue have extraordinary revenues, which are right behind the revenues from fees and can be considered as an essential component in the financing of NPs.

Based on all the above, research suggests that in the future fee revenue have the greatest potential for development and its diversification can improve businesses of NP, especially in regards to the changes in the region. Also, the analysis shows that the revenues share of grants and *Is* of grants is very small, and that in the region this revenue type is essential component in the financing PA. Therefore, one of the objectives of the policy development of protected areas, should go toward improving the mechanisms for the collection of fees stemming from tourism and recreation, which are currently neglected, and creation of a special state mechanism for funding work in national parks.

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## WATER POTENTIAL OF BEECH FORESTS IN SERBIA

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### Abstract

Hill-mountain Serbian area includes the greatest part of beech forests. They differ from each other in growth, age and structure, located alone or consisted of other trees species. The most important management goal is to produce as much as possible woody mass, but, on the other hand, generally some useful forest functions are neglected. There is not so well-known aspect of hydrological forest function that emphasizes the importance of beech for water existence. It attracts a big attention especially in the time when supplies of water are lacking so much. Some modern researches predict increasing that role of beech forests, because some big supplies of high quality water will be located in forests region, especially in its beech parts. There is analyze based on available dates (distribution of forests depending on heights, rainfalls depending on zones, coefficient of water running, etc) that is performed in the scientific paper in order to evaluate benefit of useful waters from forest ecosystems that belong to mountain-hill area of Serbia.

**Key words:** hydrological function, water, natural supplies, beech forests, benefit of useful waters etc.

### Introduction

Water presents one of the most precious natural values and due to its importance, it is increasingly becoming very important for strategic purposes. Greater number of inhabitants, quick development of industrial production and big water demands of agriculture are main characteristics of current century, so it means a big lack of not only drinking water, but also of water for industrial purposes. All predictions about bigger water needs are surpassed, so current needs for it are about 5130 m<sup>3</sup> per year. The most water spends agricultural production (about 69), then follows industry with 23 % and households at the end with 8 % (Velašević V., et al., 2002.).

Image 1: Beech and fir forests of Goc mountain

Problem of small water quantity is very serious because of global climate changes such as global warming, which means that people need to take more water. The other big problem is connected with pollution that endangers not only water on the ground, but also underground water. These all are reasons that endanger supplies of good water quality in the world. The aim of this paper is to emphasize all problems of water providing in Serbia and to suggest some ways of using it in mountain - hill forest regions.

### **Object and research methods**

As for structure of land using, Serbia belongs to agricultural countries and the whole surface of forests includes about 29 % from the overall surface (graphic 1), which means that it belongs to mid-forest-developed countries. The whole surface that includes forests is about 2 252 000 ha and about 53 % are state forests and the rest of 47 % are private forests. As for quality structure, there are about 64.7 % of coppices, natural forests make about 27.5 % and then follow artificial structures with 7.8 %. As for species, beech dominates with 29.3 % and there are many different types among it, from oak and beech (*Querco-Fagetum typicum*) forest to typical beech mountain (*Fagetum moesiaca subalpinum typicum*) forest. As for quantity of useful good quality waters, beech forests are the most desirable plant associations.

### **Graphic 1: Area structure by land use**

The importance of vegetative cover is related to retential abilities of water running, then in keeping possibilities of big water quantity and the presence of it is very important and has the greatest influence to form of hydrological processes at some catchment area. The greatest impact on water cycle in nature belongs to wood, because it presents the most stable ecosystem due to its spatial distribution and complexity. Beech forests come to the first place – these are plant associations with the greatest effect of mediating between rainfalls and water running. In this scientific paper, there are many ecological, hydrological, demographic and other research methods, so there are many analyzes based on it and sinthesis performed for evaluating overall beech forests potential and its importance for useful waters producing.

### Results of research and discussion

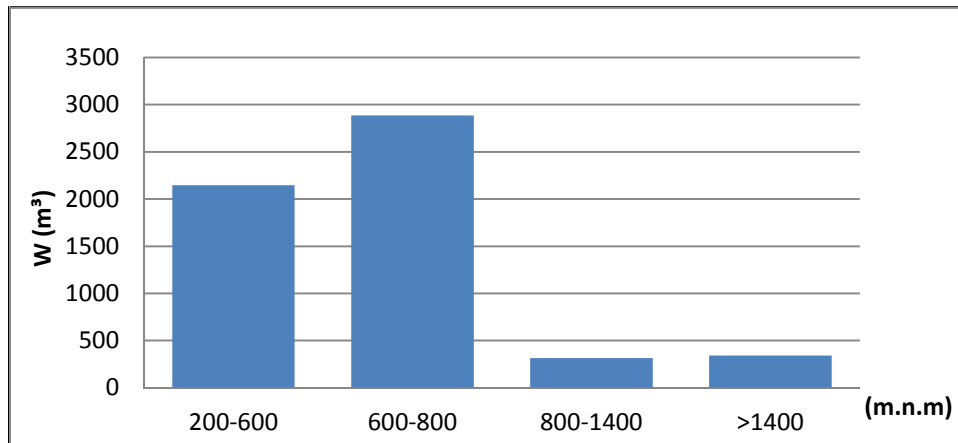
Catchment areas are characterized with regimes of water running, benefit and water quality, and aquatic systems have also some specific biological, ecological and physical characteristics that depend a lot on ways of land using. Planning of land managing at catchment area is a base for planning of water managing, but first of all, it is a base for useful waters benefit that are necessary for industry, agriculture, etc. Mountain-hill area of Serbia is covered with forest vegetation, so therefore it presents resource of great quantity of high waters quality, which is very endangered resource. There aren't some exact measurements of water quantity, that forest ecosystem keeps and distributes in time and space, so because of it, the evaluation of forest state was done. It was done on the base of some available dates about height-distribution of forests, then on the base of average rainfalls depending on heights and it was result of not only domestic, but also foreign researches (Lj. Lj., r. vic ., 1995.). The analyze of these dates was shown in a table 1 and it's very indicative, even though there are only approximative dates about water running and state of water resources in that area.

Table 1: Evaluation of water running from forests of Serbian mountain area

Numb.	Forest associations	Quantity of woods (10 <sup>3</sup> ha)	Heights (m.n.m.)	Average rainfalls (mm)	Water running			
					Coeff. of runoff	Specific runoff	Overall quantity of running water	percent
						q (l·s <sup>-1</sup> ·km <sup>-2</sup> )	W (10 <sup>6</sup> m <sup>3</sup> ·god <sup>-1</sup> )	%
1	Oak, oak and beech and other hardwood species	1103,5	200-600	720	0,27	6,16	2145,2	37,7
2	Beech, beech and oak, beech and hornbeam and other hardwood species	945,8	600-800	825	0,37	9,68	2887,05	50,8
3	Beech-fir (spruce)	90,1	800-1400	970	0,36	11,07	314,63	5,5
4	Spruce and others Gymnosperms	112,6	>1400	1080	0,28	9,59	340,5	6
	Overall/average	2252		792	0,32	8,01	5687,38	100

The overall benefit of useful waters from forests of Serbian mountain area is evaluated on about 5687.38 millions m<sup>3</sup> and the greatest role in this amount plays area located on heights between 600 and 800 meters, where are beech forests, alone or in associations with oaks and other Angyosperms (tabela 1, graph 2.)





Graph 2: Diagram of volume of water running from mountain-hill area of Serbia

The lowest area (200-600 meters) is the most widespread, because it covers about 50 % of the whole surface, but it includes just 37,7 % of the overall water quantity, which is expected from the space where are not only forests consisted just from oak trees, but also this one with other species. The area with beech and fir forest give some special hydrological effects, but it covers just about 4 % from the whole surface and has a small participation in quantity, which is about 5.5 % from expected water benefit.

On the base of ground water management GWM (1993.), Serbia has about  $5\,670\text{ m}^3\text{s}^{-1}$  or  $178\,662 \cdot 10^6\text{ m}^3$  water, and 91 % from it are international waters and just 9 % belongs to domestic waters (table 2).

Table 2: Structure of available waters in Serbia, \*GWM (1993.)

Surface $88.361\text{ km}^2$	flow $Q$ ( $\text{m}^3 \cdot \text{s}^{-1}$ )	runoff water volume $W$ ( $10^6\text{ m}^3$ )	rainfalls $h$ ( $\text{mm god}^{-1}$ )
The whole amount of available waters	5.672	178.662	2.022
International waters	5.163	162.635	1.841
Domestic waters	509	16.027	181

\*GWM- Ground water management of Serbia

These supplies of waters in Serbia are very small and with its specific water running of  $5.7\text{ l}\cdot\text{s}^{-1}\text{km}^{-2}$ , provide just  $1500\text{ m}^3$  per inhabitant a year, what means that Serbia belongs amongst the poorest European countries, because just area with  $3000\text{ m}^3$  of it provides enough water quantity (B.,(1993.)). The observation of water potential from forest area (about 29 % from the whole surface) is very logical, because about 35 % of high quality domestic waters can be expected from that area (Nikic Z., et all, (2012.)). Some useful forests functions such as hydrological, erosion protective function, put beech forests at the first place, because it protects land with its construction in the best way (Lj., et all (2011.)). In that, not damaged part of land, there are many bio-chemical processes which provide big quantities of high water quality, forest ecosystem introduces a specific bio-cleaner, as well (Nikolic V., et all (2012.)).

### Conclusions

As for possibilities of forest ecosystems, there are many advantages of it such as:

- Acceptance of big quantity of rainfalls and its further distribution without destroying of land. Water retention is a famous effect and has a great influence on spatial and time distribution of water in catchment area.
- Big forest lands abilities of infiltration of forest lands decrease harmful water running and increase underground water moving.
- There is a big hydrological importance of beech forests, that cover about 29 % from the whole surface of forest area and have a great influence on a hydrological net of mountain-hill forest area in Serbia. It produces big quantity of high waters quality. of mountain-hill forest area
- Finally it needs to be emphasized that planning of forest managing (ways of using woody mass, roads-building, providing of best hydrological structure) presents planning of water managing at the same time.

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## NATURAL RESOURCES AND THE ENVIRONMENT OF UPPER DANUBE

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### Abstract

The Upper Danube area is in the northwest of Backa of Serbia along the left bank of the Danube on the border with Croatia and Hungary. This is very specific and unique complex of wetlands and floodplains in the Danube area. The area was declared a special nature reserve because of the great biodiversity which was established here. Upper Danube is the subject of strategic documents at national and international level, particularly delineated and implemented measures for the protection and conservation of biodiversity of these ecosystems is also emphasis on individual forest species, bird species and fish stocks. The aim of this paper is to analyze the environmental situation of the region with special emphasis on the individual municipalities that are related to this area. According to the available data were analyzed indicators related to the state of the forests in this area and highlighted the importance of preserving natural resources and the principles of sustainable development, which are the potential of the area in terms of tourism and economic development.

**Keywords:** Upper Danube, biodiversity, forests, sustainable development.

### Introduction

Connecting communities and nature conservation in the European Green Belt initiative is one of the first projects in coordination IUCN- Programme of Office for South-East Europe. Planned activities in the field are focused on cross floodplains bordering Croatia, Hungary and Serbia, which is one of the last major floodplains of the Danube. The main objectives are to strengthen cross-border cooperation, raise awareness in local communities about the importance of biodiversity and capacity building of all interested parties associated with protected areas in the region. In this way, the projects contributes to the harmonization of management plans in three adjacent protected areas and involve local economy management programs are one of the key solutions for a sustainable future.

Special nature reserve Upper Danube is a protected natural asset, which extends along the left bank of the Danube, from the 1367th the 1433rd km of its course. Part of a large marsh complex that extends through neighboring Hungary and Croatia, and considered as a whole, is one of the last major flood-prone areas in the territory of the European continent.

Special Nature Reserve Upper Danube declared 2001th as a protected natural area of great importance, and the first category, based on the Regulation on the Protection of the special nature reserve "Upper Danube" (" Official Gazette of RS", no. 45/01, 81/08 and 107/09). Reserve a rarely preserved marsh complex (Monostorski and Apatinski rit) with specific natural, cultural, historical, tourist, environmental and other values. Upper Danube and enjoys international protection. Because of the known ornithological value of this natural resource is identified as a Bird Area (IBA) and the plants and butterflies (IPA PBA). Upper Danube was registered in the Ramsar list as a significant wetland according to Ramsar Convention. Upper Danube is a part of the Emerald Network, which covers areas important for the protection and conservation of wildlife species and their habitats, and a potential reserve of Biosphere (UNESCO-MAB).

In the northwest part of Backa, on the left bank of the Danube, bordering with Croatia and Hungary, there is rarely preserved flooded complex, irregular and elongated as authentic testimony original nature, special nature reserve Upper Danube. Along the neighboring Croatian coast stretches Nature Park "Kopacki rit", while to the north, the Special Nature Reserve bordering the Upper Danube Drava National Park and makes them one of the largest wetland whole of Europe. Special Nature Reserve Upper Danube includes two big marshes - Monostorski and Apatinski, which together make up 19 605 ha of forests, meadows, ponds and wetlands, including the river Danube and its meanders. The special nature reserve Upper Danube is present 51 species of mammals, 248 species of birds, 50 species of fish, 11 species of amphibians, 9 reptiles, large numbers of invertebrates, especially butterflies from over 60 species of butterflies. There are more than 1000 species of plants. With a variety of swamps, and canals rich in flora and fauna, the area of the Upper Danube region is ideal for variety of recreational and tourist activities.

The EuroVelo project "EuroVelo" is a project of the European Cyclists' Federation (ECF), through which developed network of bicycle routes of Europe. It consists of 12 trans-continental routes that go all over the continent with a length of over 60,000 km. Route "Euro Velo 6" connects the Atlantic coast of the Black Sea and beside the tracks Serbia has entered the Route 11 project Route No. 6 moves from France and go through Vienna, Bratislava, Budapest, Novi Sad, Belgrade, Bucharest and Constanta. Part of this route, which follows the Danube is called "Danube cycling route" and follow the course of the river starting in Budapest and to its mouth, and the part that follows the river through our country debt is 665 km. On part of the route through the Upper Danube, from the border with Hungary and Croatia set up a complete cycle signals, signs and demonstration table, in full accordance with the European standard for labeling these cycling routes, with tourists in the EU is enjoying growing popularity (<http://www.eurovelo.org/>).

Upper Danube is a unique whole vast ecosystem of flooded forest (black and white poplar, red and white willow, the higher areas of oak, ash and other species), numerous tributaries and canals and ponds and meadows. In these living communities inhabited marsh animals: marsh deer, wild boar, fox, deer, wildcats, otters and more. Since birds can come here to meet waterfowl and eagles, as well as very rare species such as the black stork and white-tailed eagle. Ornithological importance of the Ramsar site as a habitat for many waterfowl (considered to be more than 20.000 most numerous distinguished grebes, cormorants, herons, ducks, geese, gulls and terns. Among the other species of this community are home to many fish, amphibians, reptiles and insects. The Public company Vojvodinasume - Woodlands Sombor managed and planned to have natural resource reserve Upper Danube ([www.vojvodinasume.rs](http://www.vojvodinasume.rs) access).

### **Material and Method**

Upper Danube is analyzed in this paper in terms of natural resources. A particular within the state of the environment and biodiversity considered state forest fund. The applied analytical methods were based on the collected statistical and other documented information that was available.

### **Results and discussion**

Upper Danube is a unique whole vast ecosystem of flooded forest (black and white poplar, red and white willow, the higher areas of oak, ash and other species), numerous tributaries and canals and ponds and meadows. In these living communities inhabited marsh animals: marsh deer, wild boar, fox, deer, wildcats, otters and more. Since birds can come here to meet waterfowl and eagles, as well as very rare species such as the black stork and white-tailed eagle (*Haliaeetus albicilla*). Ornithological importance of the Ramsar site as a habitat for many waterfowl (considered to be more than 20,000 most numerous distinguished grebes, cormorants, herons, ducks, geese, gulls and terns. Among the other species of this community are home to many fish,

amphibians, reptiles and insects. The Public company JP Vojvodinašume and Lumber camp ŠG “Sombor” Sombor managed and planned to have natural resource reserve Upper Danube. (Fishing Area Management Programme Reserve "Upper Danube" for 2012-2021. Year, summary, JP "Vojvodinašume" Novi Sad, ŠG "Sombor" Sombor, Novi Sad, 20012th

In the area of the Upper Danube examined the Apatin, Bac, Backa Palanka and Sombor. The Plan strategy for rural development in Serbia researched all of these municipalities and the city belongs to Region 1 - High-efficiency agriculture and integrated farming. This region has a developed economy and economic indicators point to a well diversified industry and a good economic infrastructure (Strategy Plan for Rural Development, 2009-2013., Ministry of Agriculture, Forestry and water management, Republic of Serbia).

The main objective of forest management in forest areas is sustainable (permanent) forest management, including the management and use of forests and forest lands in a way and in such an instance, to preserve the biodiversity and productivity, regeneration, vitality and potential of forests to bring the level which meet the relevant environmental, economic and social needs of the present and future generations, both at the local and national level, taking into account at the same time not to interfere with and damage other ecosystems.

The concept of spatial development of forest area of Upper Danube includes the following starting points:

- establishing zones with differentiated protection schemes;
- conservation and protection of forests and forest lands;
- improving the management of the development, protection and regulation in forest area;
- implementation of the Convention, protection standards and development of forests in forest areas, law reform, sector strategies, instruments, measures and policies to the alignment of inter-sectoral coordination and participation of the competent institution has and the local community;
- sustainable development of environmentally friendly events and activities in relation to poly-functional importance of forests;
- improve transport accessibility and connection with the environment;
- functional linkages and integration of forestry with other sectors of the economy that occur in forested areas and in this regard the continuous development;
- use of forests as a renewable energy source (multi-use forests and forest land).

(Spatial plan of special purpose special nature reserve Upper Danube, the draft plan, Novi Sad 2011<sup>th</sup>).

Table 1. Shows of the total forest area in Serbia and examined municipalities and the city of Sombor in the Upper Danube and percentage

Region, area, city, municipality	Forested area (ha)	%
The Republic of Serbia	1.962.335,00	100
Region of Vojvodina	117.091,00	5,966
Apatin	4.657,16	0,237
Sombor	7.238,38	0,368
Ba	2.978,21	0,151
Ba ka Palanka	1.277,98	0,065

Source: Municipalities and Regions of the Republic of Serbia, 2012. Statistical Office of the Republic of Serbia.

The data presented in Tab. 1 point to a small area of forest at the level of Vojvodina, reflecting the large influence of anthropogenic factors in terms of the development of agriculture in the fertile Pannonian fields. In addition to development plans at the state and local level, this fact should be taken into account in some of the municipalities analyzed in terms of afforestation reached a certain result.

Table 2. Shows the forest area on municipalities for some of the years.

Forested area per year (ha)	1979.	2005.	2008.	2011.
Municipality				
Sombor	7.076	6.644	6.626	7.238
Apatin	2.984	4.877	4.350	4.657
Ba	3.140	4.114	4.616	2.978
Ba ka Palanka	2.810	10.277	4.808	1.277

Source: Municipalities in Serbia in 1980., 2006th, 2009th, and Municipalities and regions in Serbia, 2012. Statistical Office of the Republic of Serbia.

Sombor is a city in their territory of 1.178 km<sup>2</sup> has 7.238 ha of forest (which represents 6,14%). Part of the town is the Upper Danube Nature Reserve. In the city of Sombor, great attention is paid to the greening of the city and is considered the center with a relatively low level of threat. The problem of forest cover in Sombor ( which is presented of inadequate maintenance of forests and uncontrolled logging) to be resolved. According to documented plans, measures to protect plant and animal species through the following activities:

- control of hunting and fishing
- controlling deforestation
- planning and raising forest plantations and increase forest coverage from 5 to 10%.

Special emphasis is placed on the protection of sensitive ecosystems such as wet meadows, salt marshes, wetlands, steppes and the like. Also in terrestrial habitats are plowing land and conversion of arable land had reduced suppression and disappearance of authentic biocenosis.

Apatin municipality has under management (of the total 332 km<sup>2</sup>) 4.657 hectares, which is 14% (below the national average). A special contribution to biodiversity of this municipality is famous Apatinski rit.

Bac is a municipality in the observed area, which has 8,15 % of its territory covered with forest (total area of 36.500 ha of forest is 2.978 ha) (Municipalities and regions in Serbia 2012). Largest forest area is in the southern and western part of this municipality. The largest area of forest is in the southern and western part of the municipality. From represented dendroflora most American ash, poplar domestic, acacia, oak and oak as well as natural communities, and has a plantation forest. Forests are being built here to protect against flooding and strong winds. The alleys outside the forests have also erected along the roads and on farms.

For a municipality Backa Palanka is largely under the influence of human activity changed the original natural environment and biocenosis loess terraces were turned into arable land. Some parts of the former municipalities have preserved the original appearance and preserved natural vegetation and scrub steppe grasslands, especially in the Danube area. At present hilled less forest area consisting of dendroflora locust and oak. The largest forest is a forest Popina (Bagremara). Forested areas are only 2,13 % (from 57.500 ha area of the municipality is 1.277 ha of forest).

## Conclusion

Based on the analysis of the environment of the Upper Danube region and state forests as an important factor in the conservation and enhancement of natural resources led to the following conclusions:

- Upper Danube area along the banks of the Danube preserved wetland ecosystem and it is important to preserve the biodiversity of the reserve.
- Consideration of the municipality where the reserve around in the forest areas as well as the entire territory of the Vojvodina region of deficit and the measures planned afforestation undertaken and must continue to work on their implementation.
- In the context of sustainable development and conservation of nature and Upper Danube, which belongs to Serbia, is considered and treated in the context of cross-border cooperation with neighboring Hungary and Croatia and with respect to documents and conventions relating to the Danube countries and in the context of integration into the European Union.

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## WASTEWATER TREATMENT AND ITS INFLUENCE ON SURFACE WATER IN UPPER PARTS OF JIZERA MOUNTAINS

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### Abstract

Jizera Mountains in the north part of Bohemia (Czech Republic) are increasingly the destination for visitors and tourists, who find here fun, movement and relaxation not only in summer but also in winter. Most of the visitors find some accommodation just in the center of Jizera Mountains. Therefore most of historical buildings in this area are rebuilt on recreational facilities. These objects are usually quite far from bigger village or towns, so they are not connected onto the public sewerage and they deal with their wastewater treatment individually.

This thesis is focused on two locations: Smřdava Cottage and settlement Jizerka, which are both in the heart of the mountains and deal with wastewater treatment in a similar way, but each with different results. The influences of surface water by this are monitored above and below the place, where the treated waste water is let out. This influence is monitored with measurement of physicochemical indexes of water quality both in terrain with the portable meter and in laboratory with spectrophotometry method. Readings are compared with legislation in force in Czech Republic. This project has started in March this year, so the results are not completed yet. But it is already possible to state that in settlement Jizerka the wastewater treatment is more effective and influence water quality less than in cottage Smřdava.

**Key words:** wastewater treatment, surface water, recreation facilities, water quality

### Introduction

Water is matter necessary for live on our planet and very important economic material. That's why it is important to deal with its quality in streams and reservoirs and to realize that everything passes thru the pipeline could end in a stream.

The Czech Republic is a state lays on a interface of three drainage, therefore we can say, that our country is like a "roof of the Europe". Quality of outflowing water depends particularly on natural conditions, but the main influence comes from anthropogenic activities.





**Fig. 1: Area of interest in the Czech Republic**

At the beginning of 90's of 20<sup>th</sup> century the water pollution, mainly surface water, was perceived as one of the main issues of Czech environment. Majority of significant flows belonged into the class of strongly or very strongly polluted flows. Even serious contaminations of ground water appeared. Cessation or restriction of some of big industrial productions resulted in decrease of pollution discharged from point sources. While in the first half of 90th the volume of discharged pollution decreased mainly due to a decrease of the production, from the mid-90<sup>th</sup> an effect of large-scale construction and modernization of waste water treatment plant (WTP) technology began to show, which has continued until nowadays. [5]

Notwithstanding the pollution of streams decrease, an importance of pollution by substances, which are difficult to remove, increase. Load phosphates and inorganic nitrogen, which comes from agriculture and household (mainly from washing powders), is very serious. Some of point sources without the third level of waste water treatment contribute to this too.

Not only big city agglomerations or industry areas on lower parts of the rivers pollute the streams, it starts already on upper parts. The Sm dáva cottage could be a simple of this issue. This place is situated in the central part of Jizera Mountains. This chalet is very attractive for tourists; it is secluded without connection to public sewer. It has its own treatment plant, but it not effective enough to manage higher flow during the tourism season. The river Sm dá is a recipient of the purified water, the water quality is getting worse by this.

The second locality, which was chosen for this study, is an opposite of this situation. It is Jizerka settlement, also in the central part of Jizera Mountains without connection to public sewer, but wastewater disposal is designed so that the water quality in the river Jizerka is not influenced.

### Materials and Methods

Jizera Mountains are on the north of Czech basin by the borders with Poland (see Fig. 1). It is a typical Hercynian Central Mountains, which flush with the edge of forest but with well developed montane communities and with great large-scale peats on top platforms. Industrial air pollutants mainly in 80<sup>th</sup> of previous century have caused catastrophic breakdown of both spruce monocultures and natural spruce forests on a giant area. On the other hand extensive beech forests retained on north slopes. [3]

At the turn of 1967 and 1968 protected landscape area (PLA) was declared with the area 368 km<sup>2</sup> mainly for protecting extensive forests (73 % of PLA; 269 km<sup>2</sup>). [9]

Altitude is between 700 and 1000 m asl. This country is nearly unsettled, nevertheless very important terms of tourism. Annual precipitation reaching even 1300 – 1800 mm is one of the highest in the Czech. The average annual temperature in 789 m asl is + 4,4°C. Surface water is acid, low mineralized with higher content of aluminum. This area is situated on the watershed of the rivers Elbe and Oder. This territory consists of Krkonoše-Jizera Massif. The main rock is granite (simple Liberec granite).

Terrain is ragged, mostly with large gradients of slopes. Forrest soils are partly waterlogged and peaty. In Jizera Mountains there is water naturally acid and mineralized. But increased deposition during 80<sup>th</sup> negatively influenced water quality. [1]

**Sm dava** is a name of area around the same name chalet in Jizera Mountains in 847 m asl (see Fig. 2, 3). It is a place of confluence of rivers Bílá, ěrná and Hn dá Sm dá (White, Black and Brown Sm dá) and therefore of emergence of the river Sm dá. The chalet Sm dava with a bigger part of parking places around belongs to the municipality Bílý Potok (White Stream). North of the chalet there is a group of houses, which belongs to the Hejnice town.

The river Sm dá stems in the east part of Jizera Mountains; near the village Ves it crosses the state border with Poland in 209 m asl. Catchment area is 273,8 km<sup>2</sup>; length of the river is 45,9 km, average flow on the borders is 3,61 m<sup>3</sup>.s<sup>-1</sup>. [6] Water quality in this river is influenced by the lack of solutions of wastewater disposal in chalet Sm dava. Nowadays a new project of construction of treatment plant is now approved. This project should be realized till the end of 2013, it will cost roughly 1,5 mil. CZK

The first mention of settlement **Jizerka** is from 1539, when it was the seat of fowlers. Later lumberjacks and collectors of precious stones came. In the mid-19<sup>th</sup> century two glassworks were established, one of them is still working. The same name stream flows through the settlement as well as stream Saphir, where were deposits of precious stones (sapphire, ruby, chalcedony, topaz). [4] About 10 residents live there permanently.

Jizerka is exceptional by its altitude, which is 850 – 900 m asl. The combination of the altitude, relief of the valley and the river Jizerka supports the extreme weather in winter time. [4] The maximum snow depth 29. 4. 1944 was 315 cm. Instantaneous measured value -36°C appeared several time. Temperature under the freezing-point occurs even in the summer. [1]

Geografická mapa Libereckého kraje  
Geographical map of the Liberecký Region



Fig. 2: Area of Liberec Region with marked area of interest (yellow stars)



Fig.3: Location of sampling water (stars) (www.mapy.cz)

Due to convenient location the settlement is an often destination of variety recreation activities. Accommodation holiday homes gradually adapted to this fact. Thanks to the distance from other municipalities this settlement is not connected to the public sewerage system. Wastewater of

permanent settlers is accumulated into the endorheic sink with subsequent removal to the treatment plant in Tanvald (15 km far). Other wastewater flows after individual pretreatment in septic right to the river and very often to the soaking. Mountain Ranch Jizerka in the middle of the settlement has its own domestic WTP with 13 biodisks for 130 population equivalent (PE). [7]

On these localities sampling is realized monthly. Water samples are taken above and below expected sources of pollution (see the Fig.: 3). The most important chemical parameters of water quality are a scope of the analysis (COD, total nitrogen, total phosphorus, nitrous nitrogen, ammonia nitrogen, chlorides, sulphates, iron, manganese). These are determined by spectrophotometry methods. Other indicators are set in the field using portable multimeter HQ30d by Hach-Lange (temperature of water, dissolved oxygen, conductivity and pH).

### Results and discussion

How Sm dava so Jizerka are situated on the upper parts of rivers in the top part of Jizera Mountains. That's why the surface water has very high quality, this is seen in results from places above recreational facilities, where values measured are under the limits set by the law (see Fig.: 5; 6)

PH-value, which is nearly in all of cases on both localities under the limit given by law, is a result of presence of large peats, which are typical for this area (National Nature Reserve Jizerka peat). As Kulasová sais (2006), water in Jizera Mountains are naturally acid and low mineralized. Significant acidity of Jizera Mountains' environment was caused in 80<sup>th</sup> thanks to acidic atmospheric deposition.

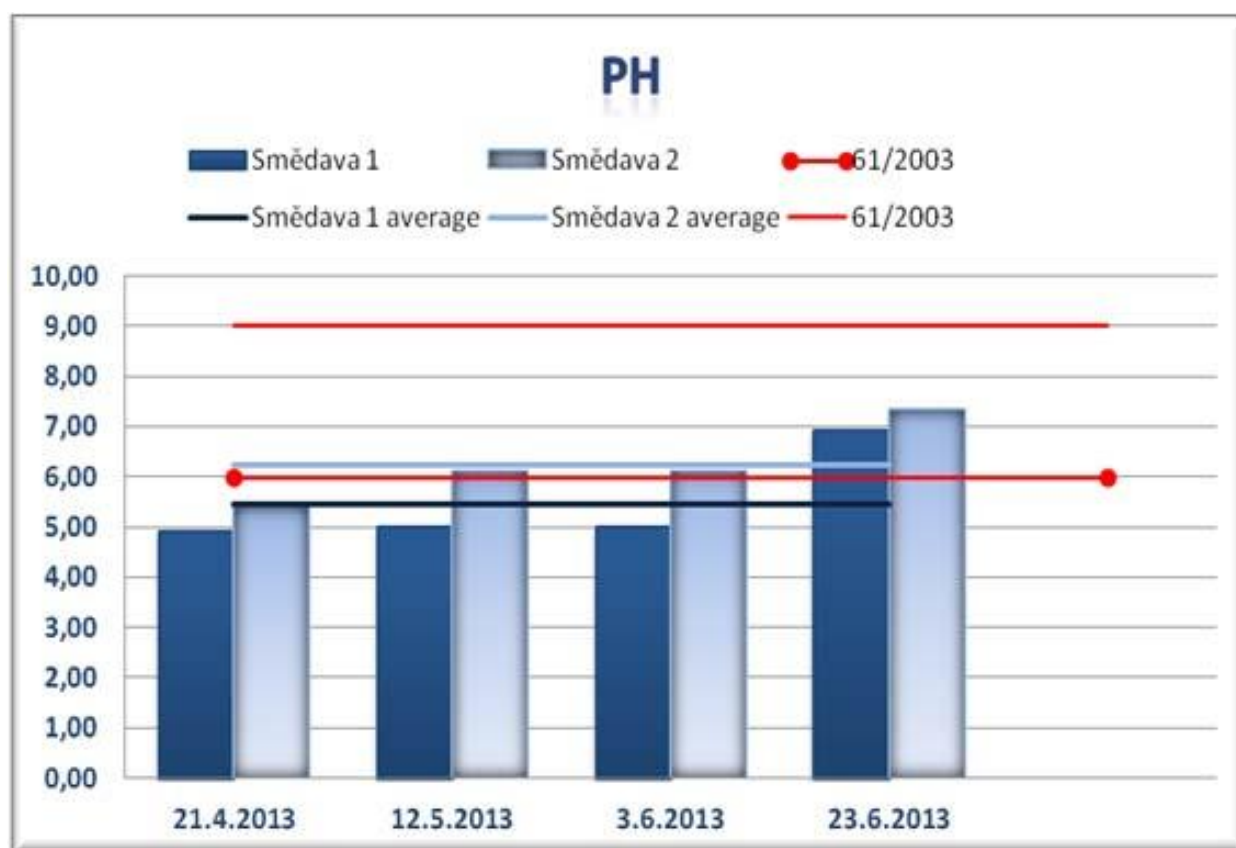


Fig. 4: pH measured on Sm dava, its average values and limits set by the legislation [2]

As results in graphs show, presence of recreation facilities has some influence on the water quality. The Fig.: 5 and 6 show increased level of some substances in March and June. High flows are cause of this phenomenon. Substances which were accumulated in the snow cover came to the flow

during the spring snow melting. Prolonged rain during June caused increase of river level. These intensive rains washed substances out of the soil.

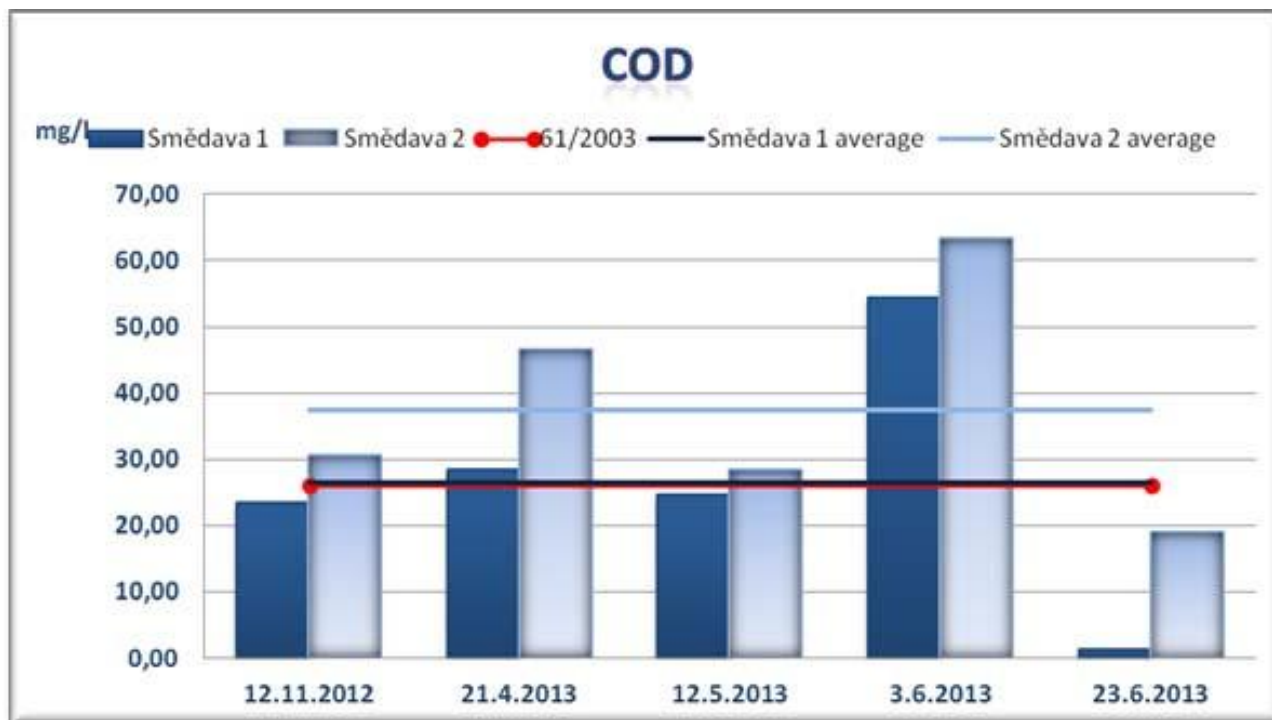


Fig. 5: Chemical oxygen demand, its average values on Smědava and limit set by the legislation [2]

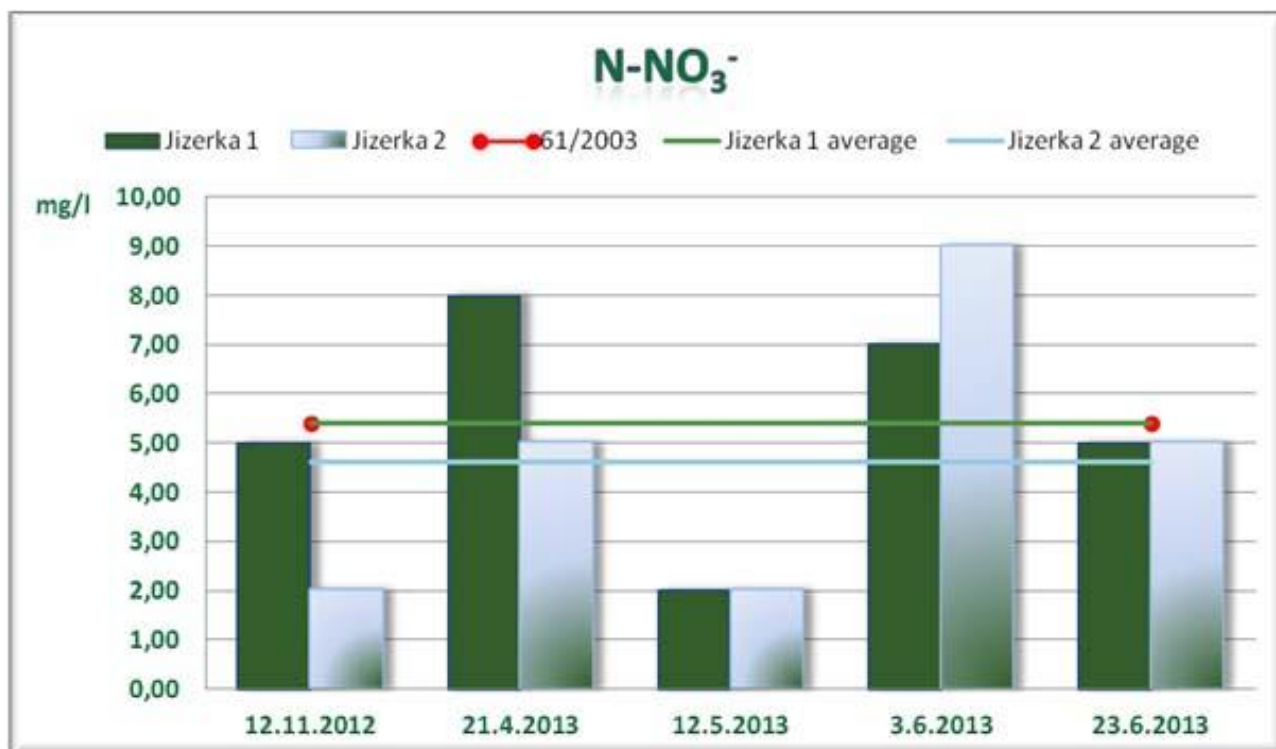


Fig. 6: Nitrate nitrogen in Jizerka, its average values and limit set by the legislation [2]

Table 1 shows a change in parameters measured above and below recreational facilities.

**Tab. 1: Percent change of water quality parameters above and below the recreational facilities**

Parameter	% of change		Distinktion Sm dava - Jizerka
	Sm dava	Jizerka	
<b>P total</b>	24,1	16,0	6,1
<b>PO<sub>4</sub><sup>3-</sup></b>	27,1	8,9	18,2
<b>COD</b>	41,7	9,0	32,0
<b>Mn</b>	25,5	6,0	19,5
<b>Fe</b>	-14,0	20,2	-34,2
<b>Cl<sup>-</sup></b>	34,7	7,2	27,5
<b>N total</b>	-38,0	-28,2	-66,2
<b>NO<sub>3</sub><sup>-</sup>-N</b>	-21,7	-14,8	-36,5
<b>NH<sub>3</sub>-N</b>	17,4	12,2	5,2
<b>O<sub>2</sub></b>	6,6	7,8	-1,2
<b>T</b>	-2,7	14,0	11,3
<b>Conductivity</b>	-16,0	-0,2	-16,2
<b>pH</b>	14,0	3,4	10,6
<b>Average</b>	7,59	4,73	-

As you can see in Tab. 1 the average percentage change in measurements is higher on the Sm dava locality. The highest change on both localities is in content of total nitrogen (38 % in Sm dá River, 28,2 % in Jizerka River), in both case it was lower below the settlement facilities. The highest difference between both localities is in the value of percentage change of COD (chemical oxygen demand), which indirectly shows the amount of organic and inorganic contaminants, dissolved or suspended in water. So this comprehensive indicator shows the best that on Sm dava there is the influence on water quality much higher there on Jizerka. Although on Sm dava there is just one house and on Jizerka there are about 35 houses.

Consequences of water pollution or differences in water quality are visible to the naked eye. There are changes in vegetation on the river banks on Sm dava from leaved grasses and bog species to broadleaf herbs like species of genus *Cirsium* and *Urtica*.

### Conclusion

In conclusion we can say, that results of physic-chemical analysis and measurements confirmed findings of field survey. Changes in vegetation around the water flow from chalet Sm dava and growth of algae on stones in the river basin were determined during reconnaissance. Ammonia odor is felt far from the end of pipes from chalet. Contrast, nearly no similar changes are on the second locality, Jizerka.

Proposed large-scale WTP for chalet Sm dava should solve this issue. Regular sampling and measurement is planned even for future, so it could be assessed real mitigate of negative influence of the recreational facility on the water quality in Sm dá River. This is very important not only because Sm dava is in the second water source protection zone. For preservation of water quality it's very important to strict comply all principles of water protection.

Water protection in the Czech Republic is divided into general, particular and special. The general protection is based on various legislative laws relating to the protection of individual environment components. It is obligatory for all and without any compensation. The particular protection includes protected areas of natural water accumulation, sensitive areas and nitrate vulnerable zones and for compliance with farming aren't also compensation. The special protection includes protection zones of water resources.

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**EFFECT ON PH, EC AND OM OF THE USE OF URBAN WASTEWATER IN IRRIGATION LINE IN THE PADDIES IN ALBUFERA OF VALENCIA (SPAIN)**

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**Abstract**

This study attempts to establish the effects of urban waste water irrigation on the cultivation of rice through pH development, organic matter and electrical conductivity along an irrigation line evaluating nine plots.

The main source of water is of urban origin (sewage) that was combined with water from irrigation ditches (several sources: agricultural, urban and industrial). This study will help determine to what extent affects the soil-water-plant systems and the surrounding environment. Study results suggest through the process of analysis of the water, of agricultural soils the impact of different types of water will have on the crops and environment. The unique aspect of this study with respect to prior studies on the reuse of urban wastewater in agriculture and their effects on crops and the environment is just how to follow the linear evolution (spatial) of the chemical parameters of flooded soils for growing rice. In the analysis not only does this consider the spatial evolution but also the temporal, comparing the results obtained from samples taken before and after harvest of rice cultivation for several campaigns. The aim is to get an overview of the effects caused by these three chemical parameters in the wastewater on rice crops, waters and agricultural soils, under the conditions of the experiment, basic soils and Mediterranean conditions.

**Keywords:** rice, irrigation, soils, urban wastewater.

**Introduction**

One of the main problems of the modern cities is how to accommodate urban residual waters and the need for recycling water. In Valencia (Spain) the urban residual waters are used in part of the irrigation of agriculture.

Its a study of a rice area in the southern of Valencia city, the altitude of the area decreases to the south of Valencia, where the depression begins and is occupied by the waters of Albufera Lake. The Albufera runs parallel to the coast from north to south over a stretch of 15 km, separated only from the Mediterranean Sea by a narrow strip of sandy soil.

This study demonstrates the effects of irrigation with urban waste water within on the cultivation of rice and in the environment (Albufera Lake). The analysis of the basic soils and Mediterranean climate and altitude conditions are the key elements of this experiment.

The soils selected from paddy fields are in the same area that is usually irrigated with treated wastewater. The objective is to quantify temporal evolution of pH, organic matter and electrical conductivity through irrigation line and through the vertical infiltration, regardless of the horizontal results which has been studied in other experiments.

**Materials and methods**

Nine plots was chosen in the area, four of which are irrigated only with water from the issuer of the treatment plant along the same line of irrigation (plots 2, 3, 4 and 5), another with water from the lake (parcel 9), one with very poor water quality of another irrigation ditch called Favara (Plot



1), another two (plots 6 and 7) where sewage water is mixed with water from the Favara and finally the plot number 8 to receive three types of water (Favara, sewage and lagoon). The irrigation line is important for the study, as may occur or not variability from one plot to another.

In this election of the plots has been taken into account its situation along a line of irrigation, but has also been chosen plots irrigated with water similar but different to compare. The sample plots were selected randomly keeping in mind the discriminating spatial variability of soil to assess, as it sought to be sufficiently separated to detect possible changes within the parameters, although other criteria were considered as part of the controlled study: the owner, etc.

Sampled soils plots were tested before and after harvest in of three soil depths. Also water samples were taken in flooded plots and at the entrance to the ditches. Sampling was repeated during three growing seasons.

Previously washed and dried in an oven beakers 50 mL required. The vessel remained in the oven for 24 hours and after weighed. Once dry the containers were weighed, 50 grams of soil were placed in the vessels. The glasses were put down inside the stove where they remained for 24 hours. The dried soil was removed from the oven and allowed to cool in a box, this allowed moisture absorption equal to an outdoor atmosphere. Once at room temperature the vessels and samples of dry soil were weighed again.

Official guidelines and methods of analysis of soil, water and plants recommended by the *Ministerio de Agricultura* for chemical analysis were used that complemented and contrasted with other methods, such as the recommendations of the APHA (American Public Health Association) in the case of analysis water.

The measurements of pH, conductivity in soil suspension are done with deionized water with mass ratio soil: water 1:5.

For pH measurement technique was used in potentiometry with the use of pH meter. For the determination of electrical conductivity (EC) technique was used the conductivity. Previously weighed 20 g in each soil sample to which was added 50 mL of deionized distilled water and stirring for 20 minutes and steeping.

Measuring the electrical conductivity is influenced by properties of solid and liquid phases of soil and provide a direct relationship with the concentration of salts thereof (Bottraud and Rhoades, 1985).

## **Results and discussions**

### **pH**

In the plots and in the ditches there is an increase in pH along irrigation line, as the pH is higher in the last areas where ditches receive input from the Albufera lake.

pH values obtained correspond to most soils considered moderately basic (7.8 to 8.5). Although there are some determinations of these values that slightly exceed in soils and other results obtained from the same soil at different times return within this range.

In this study the pH not only important in itself, but also because it will depend on the degree of availability of the evidence supplied by irrigation water. For pH values obtained the nitrogen and phosphorus are fully available to the plant, as well as calcium and magnesium. However, the availability of potassium falls between 7.5 and 8.5 pH range within most of the soils studied. Most metals for this pH range is in the form of sparingly soluble salts, being soluble at acidic pH as with iron or manganese, and is therefore unlikely to find toxicity problems in plants caused by the elements trace. Zinc and molybdenum salts with these pH values increase their solubility.

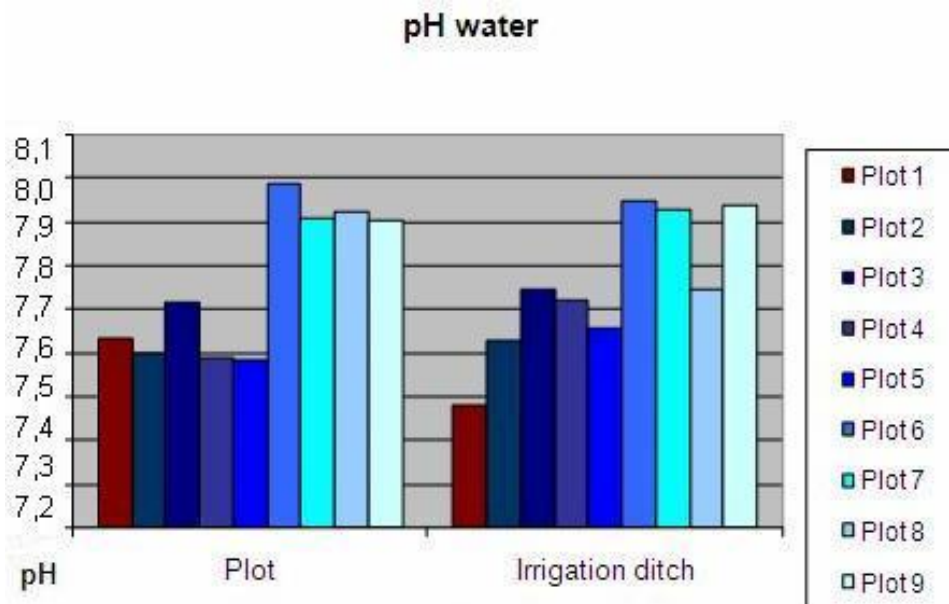


Figure 1: pH waters

The pH increases slightly within depths in most of the plots, also appears to increase slightly after harvest but this cannot establish a clear relationship as it is dependent on the time elapsed and which plots had been drained and where the samples were taken. On the other hand, does appear to show a reduction in pH as it advance along the irrigation line. The following figure displays this relationship and considers the sewage and actual distances following the route of the water to the plots.

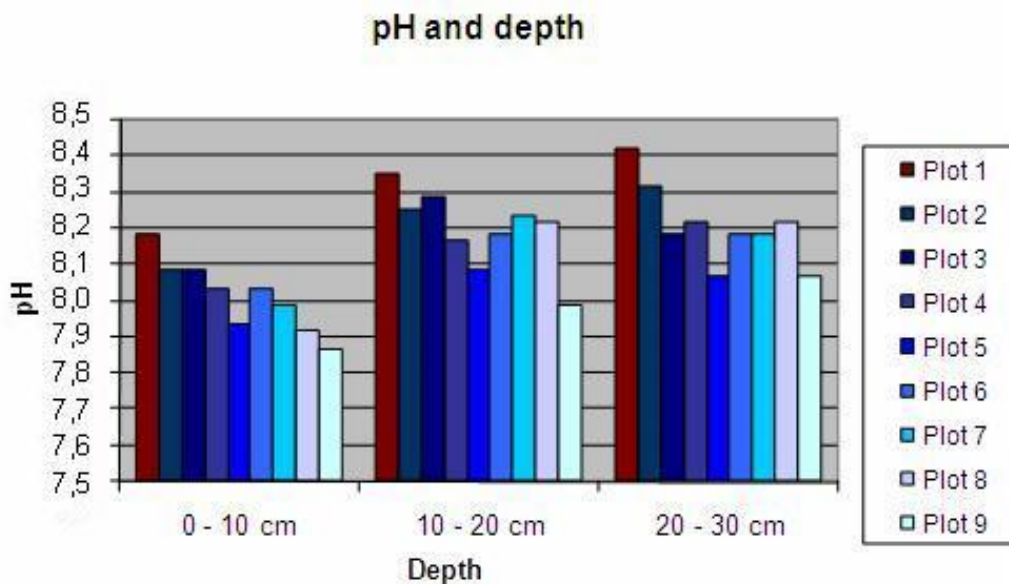


Figure 2: pH soils and depth

As shown in the picture there is a clear relationship between pH and the distance to the treatment plant along the irrigation line. Once mixed with water from other sources in plot 6 (from the red line) it seems that the pH increases again, but you can not make a conclusive statement until the results are compared from different sampling points of each plot.

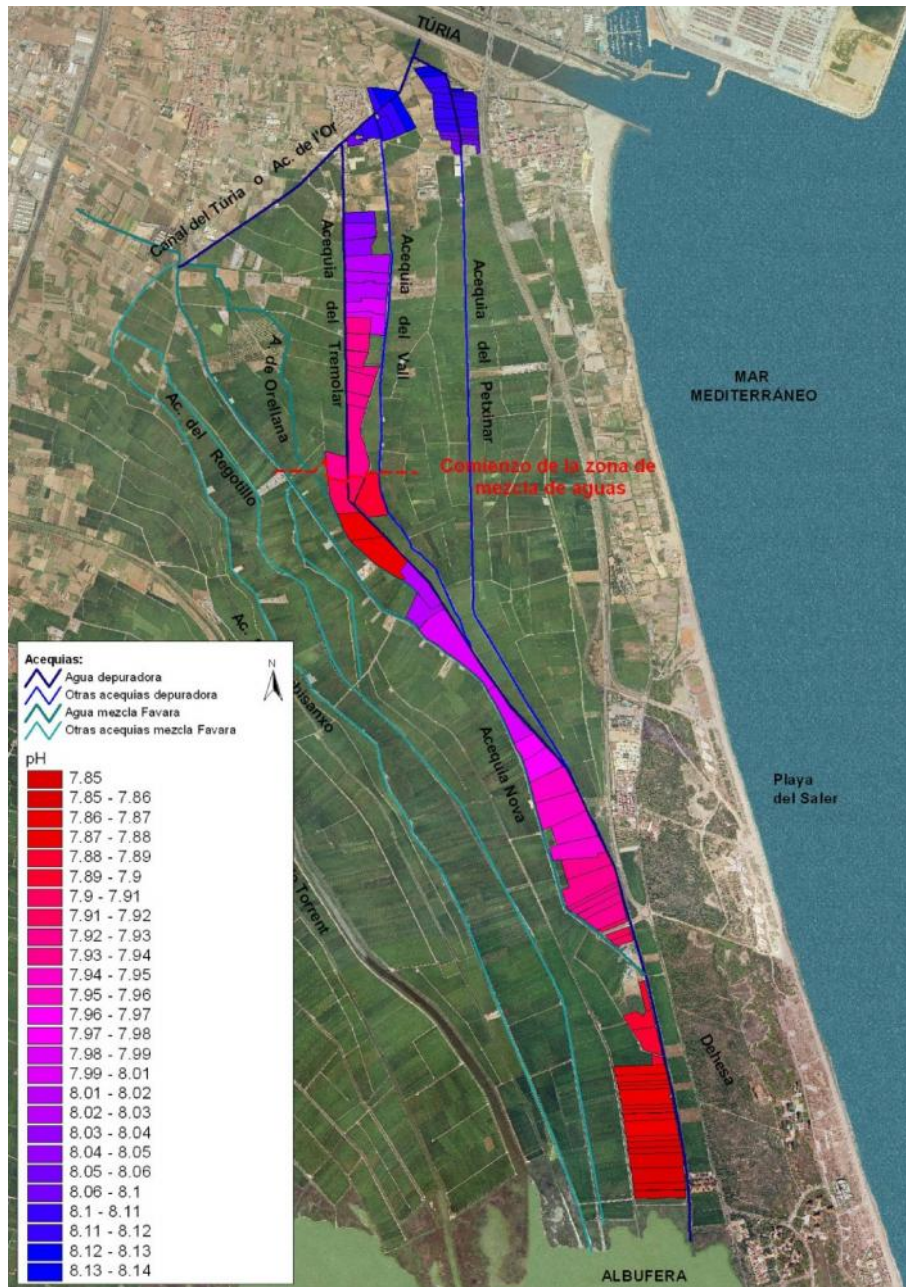


Figure 3: pH evolution along the irrigation line

### Electrical Conductivity

The electrical conductivity of the water increases along the irrigation line, in the ditches and in plots, especially from the where the waters mingle with others from diverse origins, with a peak in the plot 7.

In soil electrical conductivity was taken from the aqueous extract. As shown in the table where mean soils are increasing saline rich as in the waters along the irrigation line (with electrical conductivities of 0.5 to 2.1 dS/m). These conductivity values that are provided below are considered within tolerable salinities in soil for rice cultivation and well below toxic levels. As Perason and Bernstein point to the variety "Caloro" in the United States in are considered acceptable levels 2 dS/m and toxic from the 4 dS/m, tolerating higher levels in other phases. Furthermore, from the results it appears that there is a relationship between salinity and crop cycle time which has soil analysis.

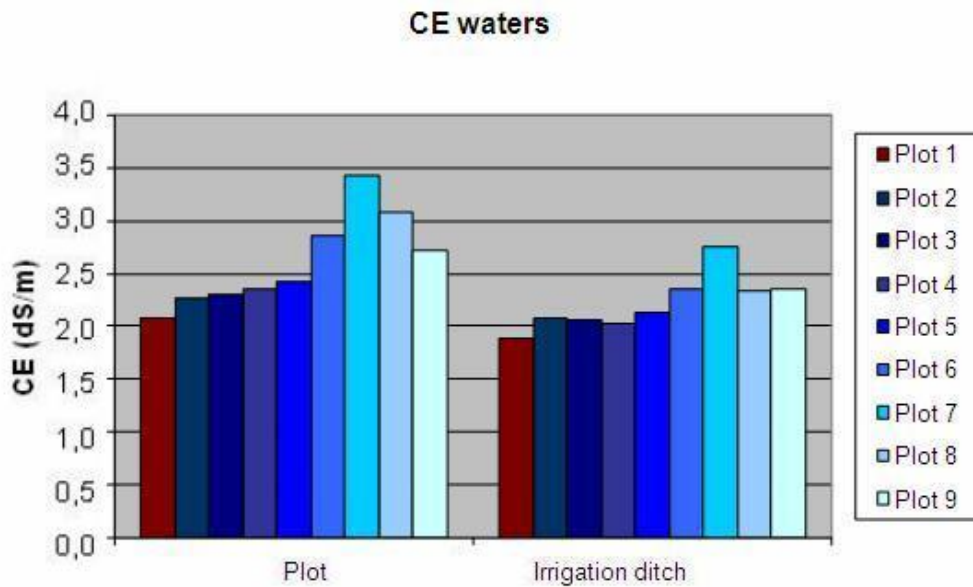


Figure 4: Electrical conductivity of waters (dS/m)

Seen that salinity decreases after harvest significantly, the more surface the sample is taken and it appears to be due to the absorption of salt by the crop roots as salinity variations are less pronounced when depth exceeds the zone of influence of the roots.

It can be said that the soils analyzed do not generally have short term salinity problems. Given its texture and electrical conductivity, the values of sodium extracted show that some may involve having a slightly characteristics of saline.

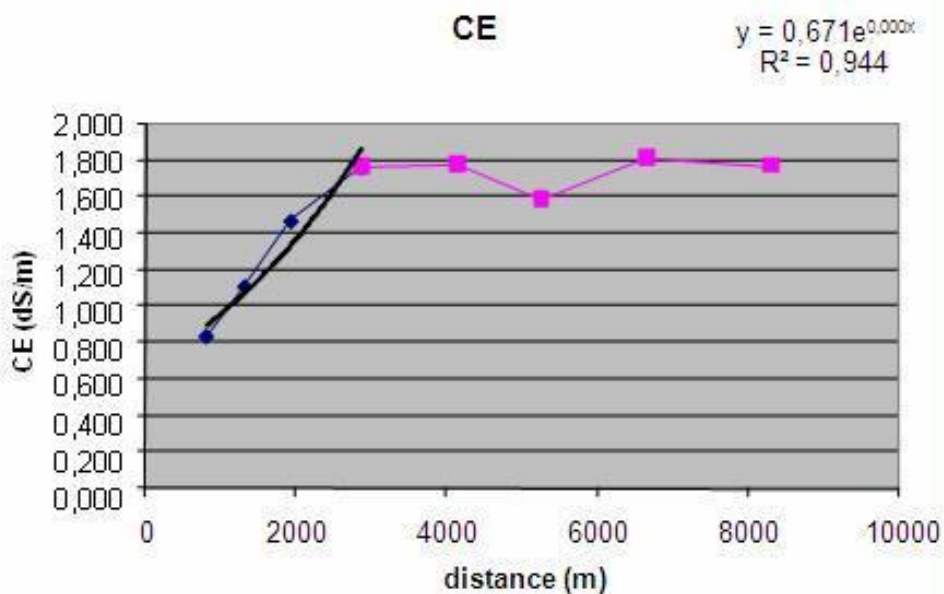


Figure 5: Electrical conductivity of soils based on the distance to the treatment plant (from plot 2 to 9)

If we relate the average conductivity in the 10 cm soil surface with distance from the treatment plant to the plot is obtained in the first four plots (with water from the treatment plant) electrical conductivity increases progressively as the distance does it then stabilized from plot 6 in which the waters of the plot are mixed with (a variant of other) waters of poor quality. (If

an attempted adjustment), the four plots receiving sewage water conductivity as a function of the distance to an exponential curve is obtained  $y=0.671^{0.0004x}$ . This setting has an  $R^2=0.9447$  which is quite acceptable, where "y" is the electrical conductivity in dS/m and "x" is the distance in meters. In view of the results of soil and water appears predominantly horizontal drag salts laundering vertically.

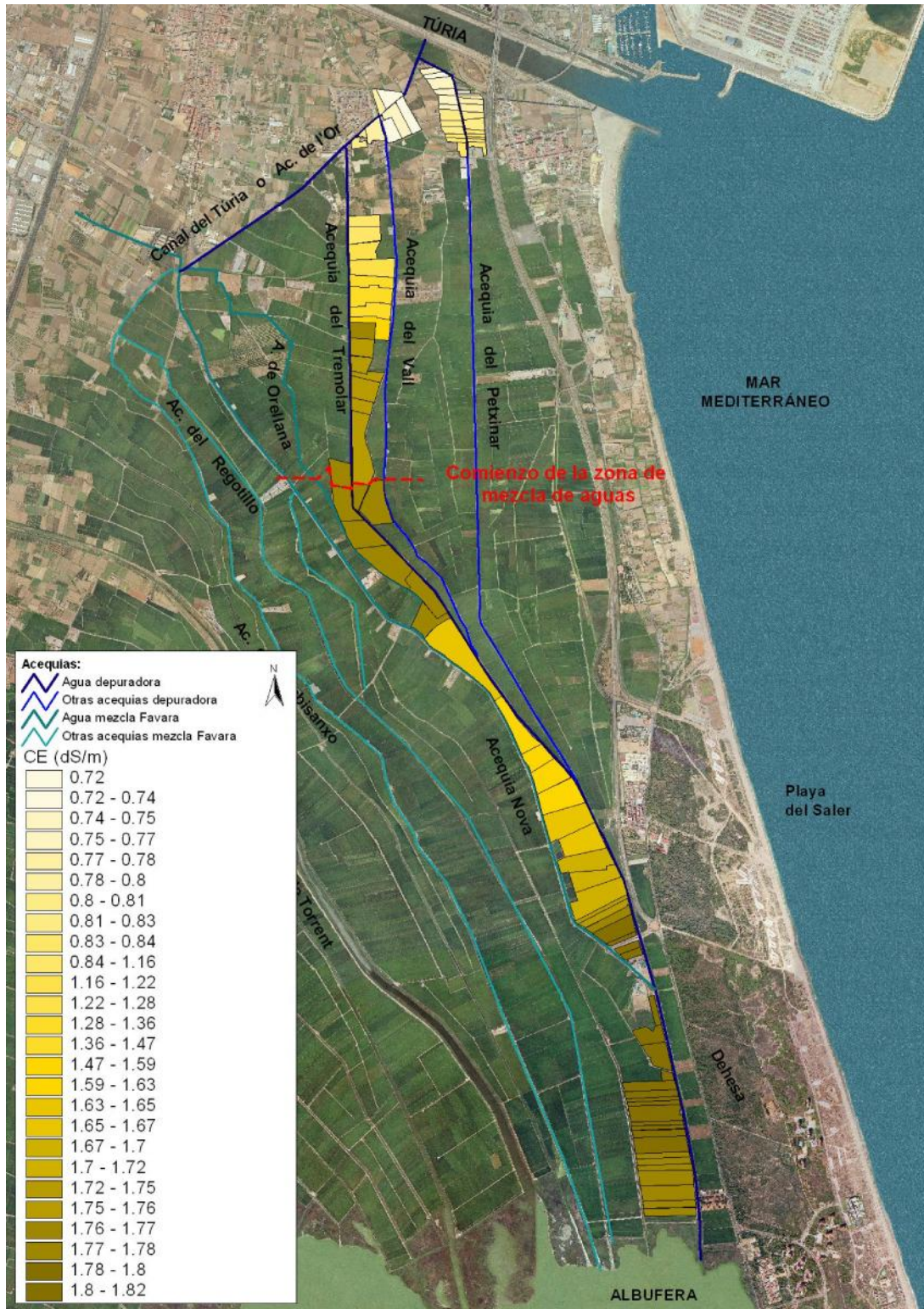


Figure 6: CE evolution along the irrigation line

Organic Matter

The results of the organic matter in the soil at the surface 30 cm along the three years of study show fields with a high organic content (of between 2.5 and 7.2%). As expected and as with the electrical conductivity, is a decrease in the percentage of organic matter with depth. Results show that matter content increases after the crop cycle organic, suggesting that the contributions are higher for this crop residues to soil losses representing consumption by plants and natural mineralization.

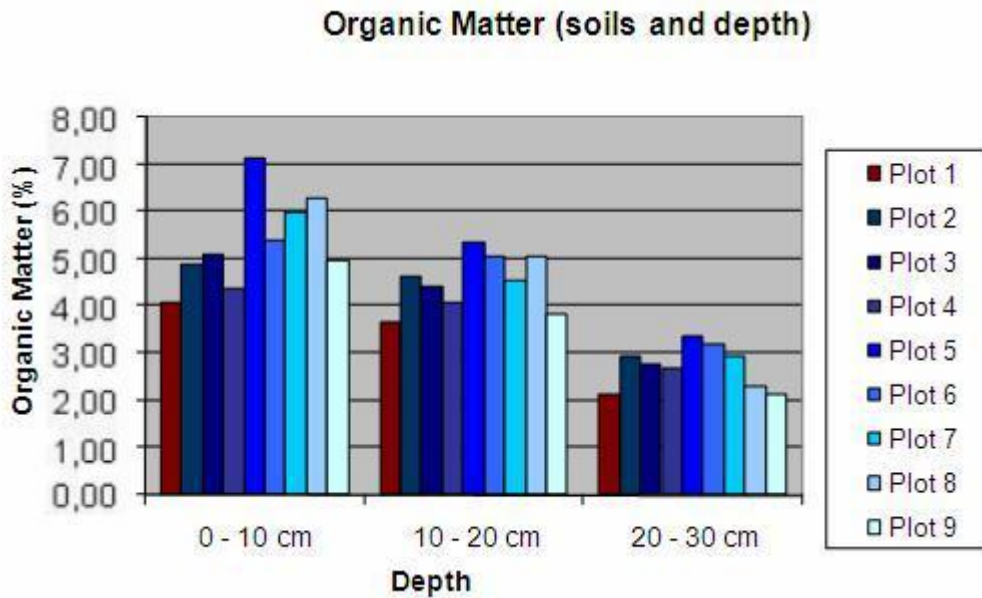


Figure 7: Organic matter percentage in the plots in each of the three depths.

An increase in the organic content by mixing the water of the first water plots from other sources that have not been cleaned, along the irrigation line there is no relationship to organic matter, increasing and decreasing without any apparent correspondence, although in each plot keeps decreasing with depth. This can be seen when rendering the percentage organic matter taking into account the actual distance of the canals that irrigate the treatment plant.

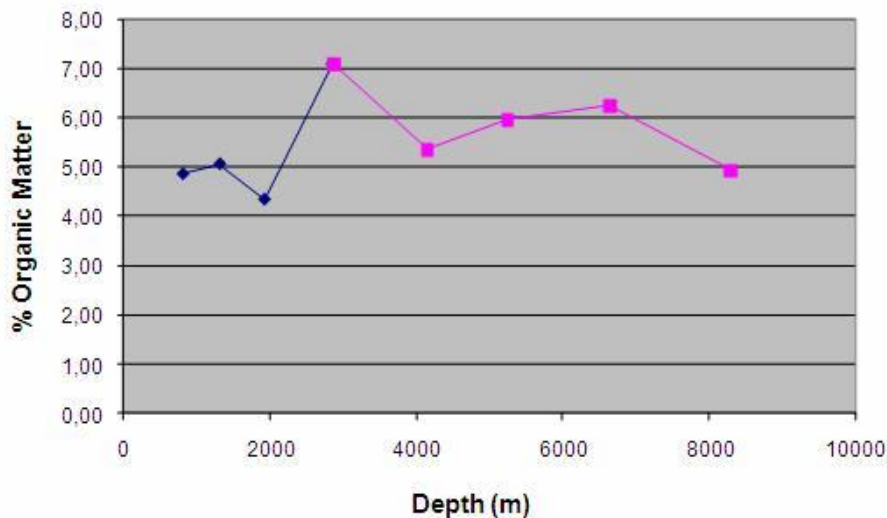


Figure 8: Organic matter percentage of based on the distance to the purifier plant exit

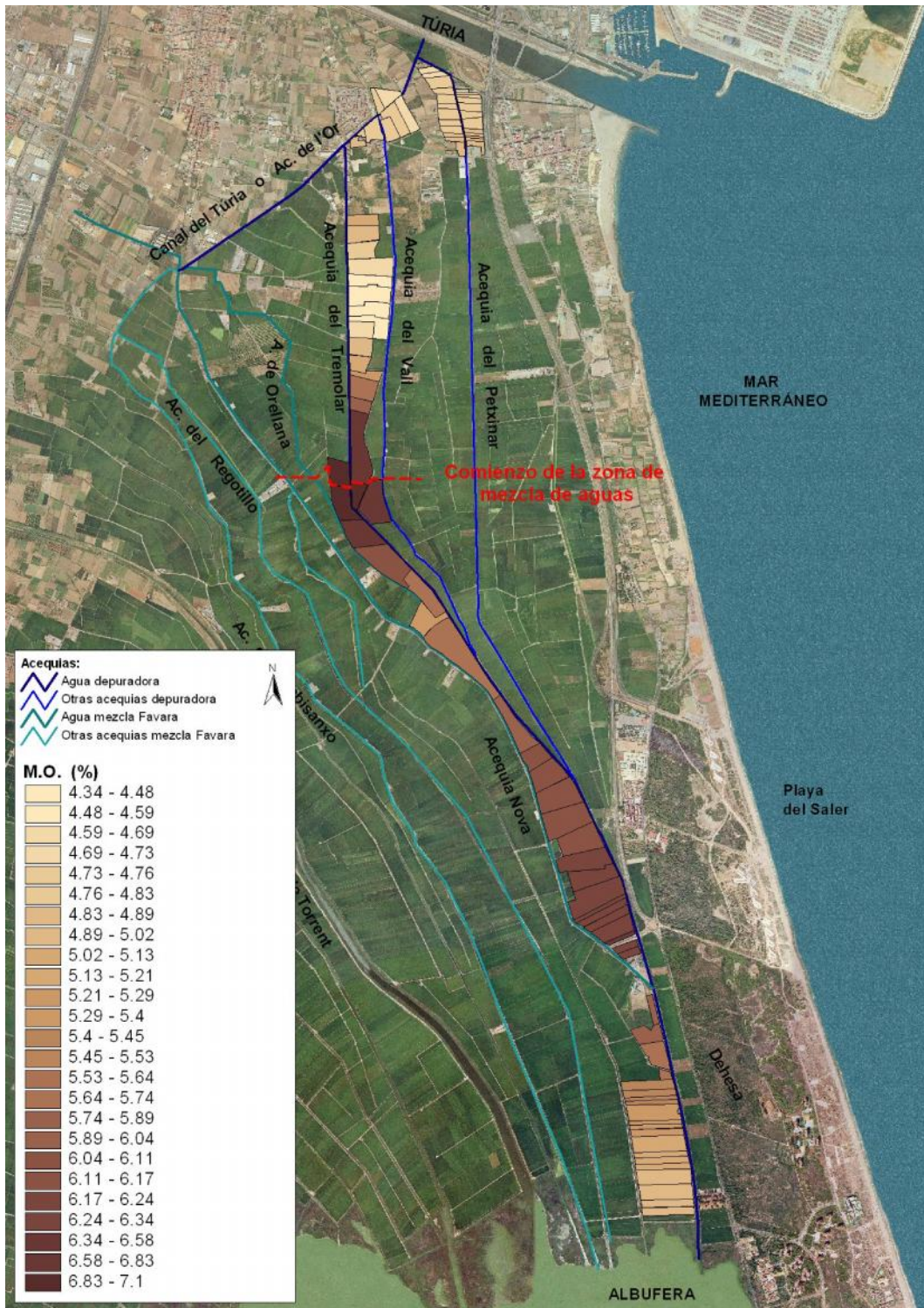


Figure 9: Organic matter evolution along the irrigation line

### Conclusions

Irrigation with sewage waters appears to slightly reduce soil pH, soils are still within normal values in the area, calcareous soils with a pH between 7.5 and 8.5. At these pH's many of the heavy metals present in these soils receiving reused water are poorly soluble salt forms. A significant reduction in pH could lead to the absence of immobilizing heavy metals in soils with a consequent increase of the concentration in the soil solution and its incorporation into the plant.

In parallel studies confirms the premise that the rice acts somewhat as a decontaminator of elements that decrease the concentration of nutrients and heavy metals in the solution of residual water irrigated soils, however, the fact that water recycles from one plot to another there is an increase in salinity along the irrigation line that involves two effects: a cumulative one plot to another and another by washing in the latest plots with the drag of solutes with soil water flooding long, it causes a salt leaching despite the low permeability soil. Still, the electrical conductivity values of soils are within the normal ranges for rice cultivation.

Sewage waters carry a large amount of dissolved salts, organic matter, nitrogen and phosphorus. The organic matter content together with high levels of phosphorus involve increased microorganisms generally that are detrimental to the crop as it is located in flooded conditions with little aerated water. These waters aren't unsuitable for irrigation, unlike present an agronomic quality than the water from other sources that carry the various canals to the rice fields of the park.

To be reused for irrigation waters discharged at the end of the process the Albufera, not only implies a better use of water resources, but also on certain parameters of toxicity levels are lower than if vertiese directly. Since the purification capacity of the soil-crop, without losing sight of the crop as a scavenger of soluble salts, organic matter and other nutrients, you might think that tertiary treatment would not be necessary as long as the water previously passed by the crop and go directly to the lake or other sensitive areas.

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## THE NEED OF SUSTAINABLE WATER USE IN TURKISH AGRICULTURE

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### Abstract

Water scarcity has been deeply influencing agricultural production over years. Continuously increasing world population and pressure on natural resources, global warming, inefficient management, and similar factors have caused individuals and governmental authorities to put more emphasis on sustainable use of natural resources, water in this case. Although the population of the world and water being used for irrigation are continuously increasing, water potential in the earth remains constant. This means per capita water consumption will diminish in the future and people will have to find out new strategies for sustainable use of water resources. Because of the sufficient surface and ground water resources in Turkey, an immediate water scarcity problem doesn't appear. However, Turkey is not counted as a water-rich country either. For this reason, the available water resources must carefully be used to provide sustainability for the future generations. The primary purpose of this paper is to develop strategies for sustainable use of water resources in Turkey. The paper is first intended to give basic information about agricultural and water resources. Then irrigation possibilities, irrigation methods, and policies will be discussed. Within the framework of the present policies and applications, recommendations for sustainable use of water resources will be developed.

**Keywords;** water sources, irrigation, sustainability, Turkey

### Introduction

Soil and water resources are the most important natural sources for all countries. Providing socioeconomic development, increasing welfare level and creating competition conditions with other communities, natural resources have a vital role. Water is one of our basic and renewable resources, but its availability is variable and limited. Surface water and ground water are both important sources not only for human use but also for ecological systems (Calzadilla et al.). Water is essential for maintaining an adequate food supply and a productive environment for the human population and for other animals, plants, and microbes worldwide (Pimentel et al., 2004).

Nearly every country in the World experiences water shortages during certain times of the year (Gleick, 1993) and more than 80 countries now suffer from serious water shortages (Falkenmark and Lindh, 1993). As human populations and economies grow, global fresh water demand has been increasing rapidly (Hinrichsen et al. 1998, Postel 1999, Rosegrant et al. 2002, Shiklomanov and Rodda 2003, Unep 2003, Gleick 2004).

Agriculture is the major user of water in the world. According to FAO (2013), at global level, the withdrawal ratios are 70 percent agricultural, 11 percent municipal and 19 percent industrial. Globally, around 50 percent of the water withdrawn for agriculture is consumed through evapotranspiration. Agriculture is responsible for approximately 70 percent of water withdrawals, but 90 percent of the water consumption (Kohli et al., 2012). In Turkey the usage of water ratios are 74 percent irrigation and livestock, 15 percent municipalities and 11 percent industrial, respectively. According to MOD (2013), Turkey is among the countries which have water constraint because of its per capita (1,645cubic meters) amount of water. In terms of renewable water sources, countries are classified in two groups; one of them is water-rich countries owning more than 10.000 m<sup>3</sup> per

capita renewable water sources, and the other one is water-poor countries owning less than 1000 m<sup>3</sup> per capita renewable water sources (SHW, 2013). In Turkey per capita renewable water amount is 345 m<sup>3</sup>/person and this number verifies that Turkey is not among the water-rich countries.

### Materials and Methods

This study was based on an extensive review of literature . The main material used included documents, reports, and statistics from the Ministry of Food Agriculture and Livestock, State Planning Organization, and State Hydraulic Works. In addition earlier journal articles, books, and proceedings were utilized.

### Results and Discussion

Turkey shows different characteristics in terms of its geographical location. Length of total boundaries is 10.765 km consisting of 2.949 km length of land borders and 7.816 km length of coastal boundaries. Turkey’s neighbors are Greece and Bulgaria from the west, Georgia, Armenia, Azerbaijan / Nakhichevan, and Iran from the east, and Iraq and Syria from the south.

### Climate

Rainfall is heavy, especially in the mountainous coastal regions of Turkey (1.000 ~ 2.500 mm / year). In the Marmara and Aegean regions; and in plateaus, and mountains of the Eastern Anatolia region rainfall is 500 ~ 1,000 mm / year. Rainfall in many parts of Central Anatolia and Southeast Anatolia is 350 ~ 500 mm / year. The Salt Lake and its surrounding areas take the least rainfall in Turkey (250 ~ 300 mm / year). However, depending on the seasons and the altitude, sufficient solar energy makes it possible to grow subtropical fruits (bananas, citrus fruits) in Turkey.

### Land Resources

Turkey covers an area of larger than 78 million hectares (783.577 km<sup>2</sup>) (Table 1). This area includes approximately 28.05 million hectares (36%) of agricultural land. Approximately 92% of agricultural land (25.75 million ha) is irrigable. According to surveys conducted by State Hydraulic Works (SHW), with the existing water potential, it is possible to technically and economically irrigate 8.50 million ha of land. Of this area 5.50 million hectares of land is presently irrigated. Approximately 3.21 million hectares of land is irrigated by SHW which built modern irrigation network in different regions.

Table 1. Land Resources of Turkey, 2013

Land Resources	Million Ha
Total area	78.35
Area of arable land	28.05
Area of irrigable land	25.75
Arid land	7.25
Economically irrigable land	8.50
Irrigated area	5.50

Source: SHW

### Water Resources

Turkey's average annual rainfall is 643 mm. This is equivalent to water by volume of 501 billion m<sup>3</sup> (Table 2). Of the total rainfall 274 billion m<sup>3</sup> evaporates from the soil-plant-water surface system and returns to the atmosphere, 41 billion m<sup>3</sup> feeds the ground water, 186 billion m<sup>3</sup> is discharged to

lakes and closed basins through streams and rivers (Kulga, 1994). Turkey is divided into 25 hydrological basins. Total average annual surface water potential is 186 billion m<sup>3</sup> and of this amount 98 billion m<sup>3</sup> can economically be developed (Table 2).

Table 2. Potential of Water Resources (Over ground, Underground) in Turkey, 2013

Average annual rainfall	643 mm/year
Total water by volume	501 billion m <sup>3</sup>
Total evaporation	274 billion m <sup>3</sup>
Infiltration to ground water	41 billion m <sup>3</sup>
<b>Surface water</b>	
Annual runoff	186 billion m <sup>3</sup>
Usable surface water	98 billion m <sup>3</sup>
<b>Ground water</b>	
Annually used ground water	14 billion m <sup>3</sup>
Total usable water	112 billion m <sup>3</sup>
<b>Water consumption</b>	
Used for irrigation (SHW)	32 billion m <sup>3</sup>
House consumption	7 billion m <sup>3</sup>
Industrial consumption	5 billion m <sup>3</sup>
Total water consumption	44 billion m <sup>3</sup>

Source: SHW

On the other hand, the total amount of underground water reserves of Turkey is 19 billion m<sup>3</sup>, and of this amount 14 billion m<sup>3</sup> of water can be pulled. The annual sum of ground water and surface water potential of Turkey is 112 billion m<sup>3</sup> (Table 2). Utilization rate of the 112 m<sup>3</sup> water sources is 39%, out of this amount 32 billion m<sup>3</sup> (73%) is used for irrigation by SHW, 7 billion m<sup>3</sup> (16%) for drinking water (16%), and 5 billion m<sup>3</sup> (11%) in the industry.

According to SHW statistics about 28.5% (52.94 billion m<sup>3</sup>) of the total 186 billion m<sup>3</sup> of usable water belongs to the Euphrates-Tigris basin (184.92 km<sup>2</sup>) which takes place in the eastern part of Turkey. This basin is followed-in area by the Red River basin (78.18 km<sup>2</sup>) and Sakarya (58.16 km<sup>2</sup>) basin. In terms of annual flow the Euphrates-Tigris basin is followed by the East Sea basin (14.90 billion m<sup>3</sup>), the Eastern Mediterranean basins (11.07 billion m<sup>3</sup>), and the Antalya basin (11.06 billion m<sup>3</sup>), respectively (SHW, 2013).

Over the last 55 years the SHW alone constructed 706 dams and ponds on the catchments, took 3.2 million hectares of agricultural land under irrigation, constructed 5930 flood protection facilities which made it possible to protect 1.4 million hectares of land, and provided 3.31 billion cubic meters of drinking, domestic, and industrial water supply services (SHW, 2013).

The gross irrigated area which was 2.3 million hectares in 1970s increased by 2.4 times and reached to 5.5 million hectares in 2011. According to SHW statistics total of 8.5 million hectares of land could technically and economically be irrigated but only 66% of this area was really irrigated by the end of 2011. Overall, 85% of the irrigation water comes from surface water an approximately half of this is provided by multi-purpose dams. In addition, the canal irrigation technology still dominates, but sprinkler and drip irrigation systems are being implemented rapidly.

Collected from an area of approximately 2.1 million hectares the SHW statistics in 2011 show that 81% of the land is irrigated by surface irrigation methods (furrow, border, and release) while pressurized irrigation systems (sprinkler and drip) is used in the remaining part. Traditional sprinkler irrigation is common and 184.000 hectares of land is irrigated by this method. From the SHW irrigations schemes about 72.000 hectares of land irrigated by drip irrigation method.

Table 3. Irrigation Areas by SHW

Status of Irrigation	Number of Facility	Net Area (ha)	Gross area (ha)
Operated by SHW	55	79.704	95.775
Operated by Irrigation Associations	740	2.209.436	2.654.934
Operated by State Production Farms and Universities	31	17.510	21.041
Operated by Cooperatives	1.384	456.709	548.250
Total	2.210	2.763.359	3.320.000

Source: SHW, 2010

As of 2010, the total number of irrigation facilities was 2.210, and 2.763.359 hectares of land had been taken under irrigation. Of this area 79.704 ha (55 facility) operated by SHW, 2.209.436 hectares (740 facilities) by irrigation associations, 17.510 hectares (31 facilities) by state production farms and universities, and 456.709 hectares (1.384 facilities) by irrigation cooperatives (Table 3).

In addition, the Southeastern Anatolia Project (GAP in Turkish), including the Tigris and Euphrates basins, particularly with a focus of energy and irrigation investments, is the region's biggest economic and social development project. Within the framework of 13 projects which are being planned under the main project of the GAP, 22 dams and 19 hydroelectric power plants with 7.490 initial mw power will be constructed in the region. From these projects a total of 27.4 billion kWh of hydroelectric power will be produced. The projects will give the opportunity to irrigate 1.058 million hectares of farmland. So far 75% of energy projects and 29% of the irrigation projects have been realized.

### Conclusion

Soil and water are considered as the most important strategic tolls of the 21st century since scarcity and famine are serious threats. Due to the rapid growth in population and booming in industrial sector, countries may face difficulties in meeting the growing need for water, and therefore water shortages will come up with water crisis in many regions in the next 20-25 years (Evsahibioglu, et al, 2010).

In the world summit of the UN Development and Environment (1992) and in UN water report of 1992 prepared for the World's Water Day, Turkey was presented among the countries where drought would be a serious problem.

According to international standards, the amount of current technical and economic available renewable water per capita per year changes from 1.500 to 1.700 m<sup>3</sup>, and these numbers indicate that Turkey is one of the countries with "water stress". The population of Turkey will reach to 80 million by 2025 (TURKSTAT, 2013), and the amount of water will be reduced to 1.100 m<sup>3</sup> per person. These values indicate that Turkey is not among water-rich countries.

There have been many problems with water use in Turkey. Among these are inefficient water resources management, climate changes, primitive irrigation methods, illegally opened irrigation wells, network losses, and insufficient waste water treatment plants, etc. In addition, the amount of water available will not be sufficient because of the increasing demand, drought, and pollution in water catchments. Moreover, issues such as lack of planning, monitoring, and evaluation; lack of control; lack of a common data base and information flow; and lack of coordination among institutions are major problems encountered in the management of water resources (Ay and Kisi, 2011). In order for Turkey to sustainable use of water resources the following recommendations can be listed;

First of all deficiencies and ambiguities in the legislation must be solved. Duties and responsibilities of the institutions must be clarified. For effective water management, coordination and cooperation among all stakeholders must be established.

An effective control mechanism for water resources must be provided. All stakeholders must take adequate measures against water pollution. Because Turkey is facing a rapid population growth more water will be needed in the future. Public must be educated to reduce water wastes. In order to reuse waste water, treatment facilities must be established (Ay and Kisi, 2011).

In order to prevent desertification in agricultural lands excessive water use with the present primitive irrigation methods must be prevented. For this reason, farmers must be encouraged, and even supported to adopt modern irrigation methods which will reduce water consumption, as well as prevents soil desertification. Water potential of Turkey must be correctly determined and sustainable use for all citizens must be ensured (MOD, 2013).

National watershed classification system should be developed so as to allow the sustainable use and protection of water resources. River basin management should be carried out by one effective organization. The working process of irrigation associations must be revised. Their system need to be more effective.

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## COCCIDIOSTATS IN POULTRY MANURE - A POTENTIAL IMPACT ON METAL ACCUMULATION IN BENEFICIAL SOIL INVERTEBRATES

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### Abstract

Carboxylic ionophore antibiotics are used in veterinary medicine for prevention and treatment of coccidiosis in poultry. The most frequently used coccidiostats in Slovenia and in northern Europe are natural ionophores monensin, salinomycin, and lasalocid. As these substances are only partially metabolised in treated animals, the major part of coccidiostats consumed are excreted in active form. When poultry manure is used as fertiliser, part of the coccidiostats ends up in the environment. Ionophores act as cation transporters across cell membranes, so they can potentially also impact metal transport, especially in metal-burdened environments. We studied the bioaccumulation of copper in earthworms (*Eisenia andrei*) and isopods (*Porcellio scaber*) when concurrently exposed to monensin and Cu-contaminated artificial soil. Copper predominantly enters agricultural soils by use of Cu-based herbicides and manure from pig farms where it is used as a growth promoter. In the present study, animals were exposed for 14 or 28 days to environmentally realistic concentrations of Cu (80 and 160 mg/kg dry soil) and monensin (25 mg/kg dry soil). We found that monensin increases the uptake and accumulation of Cu in earthworms, while no impacts on isopods were observed. Thus we may conclude that in Cu-burdened environments like vineyards, Cu intake could decrease the toxicity threshold if monensin-contaminated manure from poultry farms was used frequently.

**Keywords:** ionophore, monensin, bioaccumulation, earthworms, woodlice

### Introduction

Coccidiostats are ionophore antibiotics used to prevent and treat coccidiosis. They are administered mixed with feed in the production of broilers and turkeys. One of the most frequently used natural carboxylic ionophore antibiotics is monensin (Hansen et al., 2009a; Žizek and Zidar, 2013). In the treated animals monensin is only partially metabolised and excreted primarily via faeces, which is usually used as manure on arable land. In this way coccidiostats enter terrestrial ecosystems. When in the environment, coccidiostats undergo both biotic and abiotic degradation (Vertesy et al., 1987; Sassman and Lee, 2007; Hansen et al., 2009b; Hansen et al., 2012). The reported monensin half-lives in soil are between 2 days (Sassman and Lee, 2007) and 24.6 days (Yoshida et al., 2010). The reports on the predicted environmental concentrations of monensin vary greatly and are between 0.05 mg kg<sup>-1</sup> soil (Žizek et al., 2011) and 1.12 mg kg<sup>-1</sup> soil (EFSA, 2005).

The presence of ionophores in the environment simultaneously with Cu may alter metal assimilation in non-target organisms. Namely, ionophores bind numerous mono- and divalent cations that facilitate the passage of ions through cell membranes (Celis et al., 1974; Elsasser, 1984). Monensin is usually classified as a monovalent ionophore that combines with Na<sup>+</sup> and K<sup>+</sup> ions (Dowling, 1992) but also facilitates the transport of divalent cations like Ca<sup>2+</sup> (Ambroz et al., 1990). It has also been reported that monensin mediates the transmembrane transport of Pb in rats (Hamidinia et al., 2002; 2006).

Based on the available information on steers (Kirk et al., 1994) and chickens and sheep (Khan et al., 1993), monensin was expected to alter Cu availability to terrestrial invertebrates. Copper predominantly enters agricultural soils by the use of Cu-based herbicides and manure from pig farms where it is used as a growth promoter. Cu concentrations in pig manure can exceed 700 mg kg<sup>-1</sup> (Li et al., 2010; Xiong et al., 2010). In vineyards, after frequent and long-lasting use of Cu-based fungicides, Cu concentrations can reach several hundred mg of Cu per kg soil (Komárek et al., 2010).

In the present study, we were interested in the effects of the ionophore monensin on copper accumulation in earthworms and isopods following concurrent administration of the ionophore and metal.

### **Materials and methods**

#### Test animals

The earthworm species used in the experiments was *Eisenia andrei* (Oligochaeta: Annelida, Lumbricidae) from the laboratory culture at the Veterinary Faculty, University of Ljubljana. Sexually mature animals with a visible clitellum and weighing between 200 and 400 mg were used in the experiments. *Porcellio scaber*, Latr. (Isopoda, Crustacea), originated from the laboratory culture at the Department of Biology, Biotechnical faculty, University of Ljubljana. All tests were performed with adult animals of both sexes weighing between 30 and 50 mg. Pregnant females and animals in premoult and ecdysis period (Zidar et al., 1998) were excluded.

#### Soil preparation

The standardised natural soil Lufa 2.2 (Speyer) was used in the study. Monensin in the form of monensin A sodium salt (90% purity, Sigma-Aldrich, Germany) was introduced to the soil dissolved in acetone at a concentration of 25 mg kg<sup>-1</sup> dry soil. Soil for the experiment was spiked with the acetone solution (5 mL solution per 100 g soil), thoroughly mixed and left overnight in a fume hood to evaporate the acetone. Copper was added as water solution of Cu(NO<sub>3</sub>)<sub>2</sub>·3H<sub>2</sub>O salt (99.5% purity, Merck, Germany) in concentrations 80 and 160 mg kg<sup>-1</sup> dry soil. After application of chemicals the soil moisture content was adjusted to 60% of the water holding capacity. Soil pH was 6.5 – 7.

#### Experimental design

Rectangular polypropylene vessels (160 × 110 mm) with a volume of 1.5 L were filled with approximately 1 kg of wet soil and 20 animals were added. Animals were exposed to untreated soil, solely monensin (25 mg kg<sup>-1</sup>), solely Cu (80 or 160 mg kg<sup>-1</sup>), or to Cu combined with monensin. Altogether 240 earthworms and 240 isopods were divided into 12 exposure groups per animal species. During the exposure earthworms were fed with dry horse manure with around 8 mg Cu kg<sup>-1</sup> dry weight. Manure was mixed with soil once per fortnight. Isopods were fed with dry and partly decomposed hazel leaves with around 8 mg Cu kg<sup>-1</sup> dry weight. Leaves were renewed every 7 days. Animals were sampled after 14 and after 28 days. Ten animals from each treatment were used for metal analyses while the rest of the surviving animals were used for histological analyses (not part of this paper).

#### Metal analyses

After exposure, the animals were left on a wet filter paper for 24 hours to purge their gut. Then they were lyophilised, weighed and completely digested in a concentrated nitric acid/perchloric acid mixture (7:1 v/v). After evaporation of the acid, the residue was dissolved in 0.2% HNO<sub>3</sub>. Cu concentrations in whole animals were determined by flame atomic absorption spectrometry (Perkin Elmer AAnalyst 100).

#### Data analysis

The Mann-Whitney test was used to compare the impact of coccidiostats on metal assimilation. The decrease or increase in body metal concentrations was assigned to monensin if statistically different ( $p < 0.05$ ) from the solely metal exposed animals. All the calculations were performed with the SPSS 17.0 statistic software package (USA).

## Results and discussion

Copper concentration in earthworms exposed to solely monensin contaminated soil did not differ from control earthworms (Figs 1 and 2). In animals simultaneously exposed to 25 mg monensin kg<sup>-1</sup> soil and 160 mg Cu kg<sup>-1</sup> soil for 14 days we recorded a significantly (Man-Whitney) higher Cu accumulation compared to solely Cu-exposed animals (Fig. 1). After 28 days of exposure (Fig. 2) monensin in soil combined with Cu resulted in higher Cu accumulation in earthworms although the difference was significant (Mann Whitney test) only at the lower Cu concentration. These results are in concordance with the results reported by Elsasser (1984). In monensin-treated chickens and sheep liver concentrations of Cu increased compared to control.

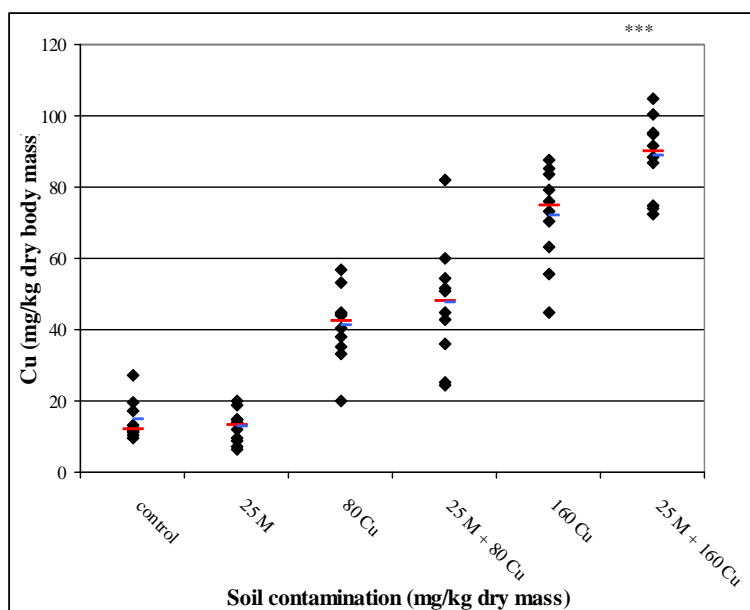


Figure 1: Whole body Cu concentration in earthworms (*Eisenia andrei*) after 14 days exposure to solely Cu or monensin contaminated Lufa 2.2 soil or concurrently exposed to monensin and Cu contaminated soil (nominal values). (diamonds – individual values; longer red line – median value; shorter blue line – average; \*\*\* -  $p < 0.001$ )

Copper accumulation in animals concurrently exposed to monensin and Cu for 28 days was in general lower compared to that after 14 days. As monensin half-life in soil at similar conditions is 23.5 days (Žižek et al., 2011), more than half of monensin probably degraded in 28 days and thus its influence on Cu accumulation was lower but still prominent. Earthworms exposed to only Cu for 28 days did not differ from earthworms exposed for 14 days which indicates that Cu body concentrations had probably reached equilibrium between Cu assimilation and excretion.



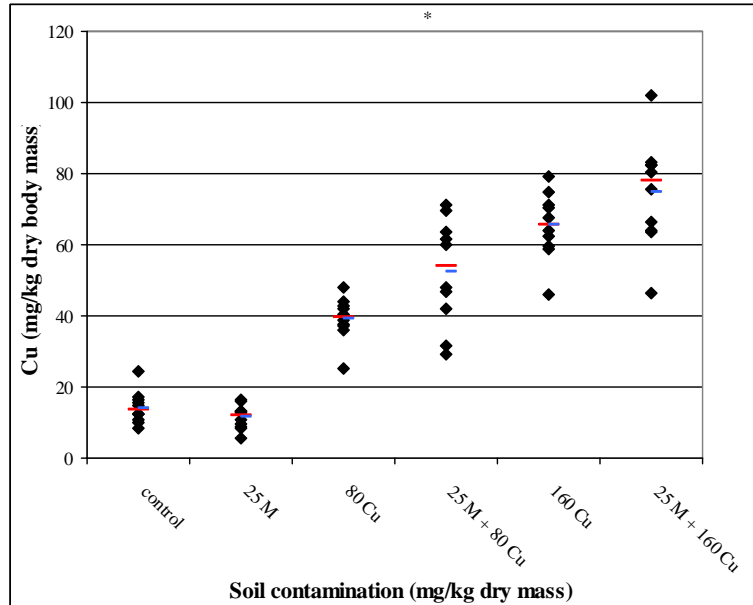


Figure 2: Whole body Cu concentration in earthworms (*Eisenia andrei*) after 28 days exposure to solely Cu or monensin contaminated Lufa 2.2 soil or concurrently exposed to monensin and Cu contaminated soil (nominal values). (diamonds – individual values; longer red line – median value; shorter blue line – average)

In isopods no influence of monensin on copper accumulation was detected. Measured Cu body concentrations did not differ between groups in spite of different copper contamination of soil. Copper concentrations vary greatly among control animals and within other groups. Whole body concentrations were also in general higher than in the study of Zidar et al. (2003), where animals were individually exposed via food. This discrepancy might be related to cannibalism as mortality in isopods was high (up to 50 %). Dead animals were immediately eaten by other animals in the vessel that thus ingested substantial amounts of Cu.

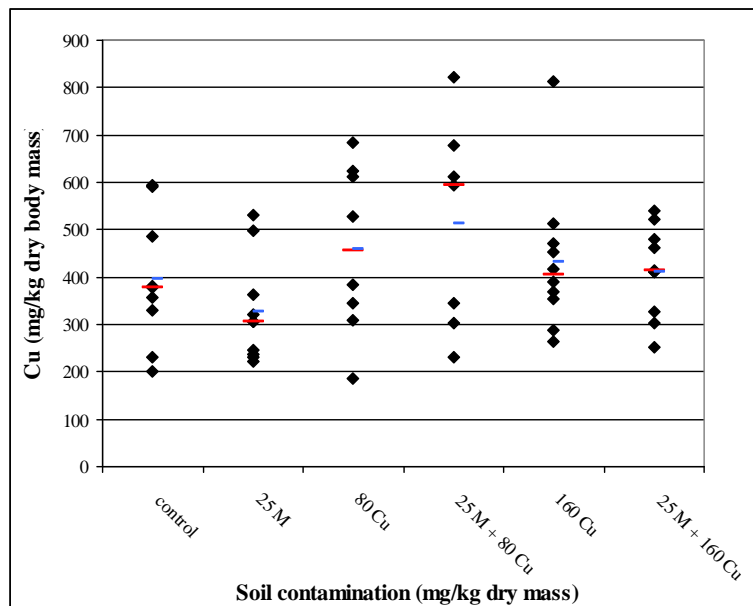


Figure 3: Whole body Cu concentration in isopods (*Porcellio scaber*) after 14 days exposure to solely Cu or monensin contaminated Lufa 2.2 soil or concurrently exposed to monensin and Cu contaminated soil (nominal values).

contaminated soil (nominal values). (diamonds – individual values; longer red line – median value; shorter blue line – average)

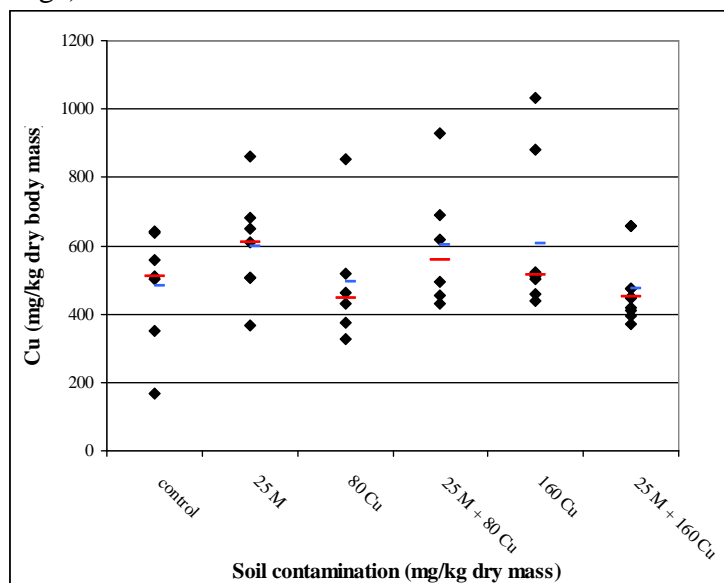


Figure 4: Whole body Cu concentration in isopods (*Porcellio scaber*) after 28 days exposure to solely Cu or monensin contaminated Lufa 2.2 soil or concurrently exposed to monensin and Cu contaminated soil (nominal values). (diamonds – individual values; longer red line – median value; shorter blue line – average)

### Conclusion

We may conclude that monensin increases the uptake and accumulation of Cu in earthworms at environmentally realistic concentrations of Cu and monensin. Therefore, frequent application of manure from poultry farms with no previous composting might increase the uptake and potentially the toxicity of metals in beneficial soil organisms. This could potentially endanger the production potential of arable soil, so care should be taken to use aged or composted manure.

### Acknowledgement

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## ECONOMIC VALUE AND PRICING OF WATER IN IRRIGATION IN SERBIA

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### Abstract

Several factors (technical, economics and social) have major influence on the price of water for irrigation. In addition to these factors and the calculation method of irrigation water prices has an impact on the amount of the same.

For regional subsystem “Srem”, which covers about 1.700 ha of arable land, different approaches of water price were investigated. Cost, benefit and marginal principle formation for irrigation of suggested agricultural plants were chosen. These methods proved that the water price is in the function of selected factors, as well as the ways of calculating the price of water for irrigation. Each of these principles enables water price calculation, which consists of water price on intake and price of water for distribution on specific farm depending on irrigation requirement.

The cost of irrigation in this case amounted to 353-689 €/ha depending from financing conditions. Economic selling price of 1 m<sup>3</sup> water for irrigation (ILRIC method) ranged from 0,29-0,52 €/m<sup>3</sup> depending from amount the discount rate (0-10%).

The obtained water price data are included in the economic analysis for individual agricultural crops on this area. It has been calculated how many tons per hectare covers the costs of irrigation. Using the binomial tariff total costs for irrigation, for the whole subsystem, are assigned to individual crops as follows: fixed irrigation costs are allocated according to the sown area (€/ha) and variable costs according to expected water consumption (€/m<sup>3</sup>). Thus farmers, which will be supplied with water for irrigation from this subsystem, will know in advance approximately the expected water price for irrigation.

The application of this methodology requires a complete monitoring by the regional subsystem, as well as the register of all users and their mutual obligations.

**Key words:** water price, irrigation, crops, distribution

### Introduction

The pricing of irrigation water (€/m<sup>3</sup> or €/ha) is a complex process as it penetrates into two sectors: agriculture and water management. Furthermore the current measures of agricultural and water policy can influence the price of water. There are numerous studies on this issue in many countries. In this sense, different methods and tariff rates models of water and water services were proposed.

Potkonjak and colleagues (2012) examined various cases of calculating the price of water for irrigation in Serbia from the local system to the multi-purpose regional hydro-systems. Depending on the type of ownership of the system authors had suggested several principles of calculating the cost of water for irrigation: cost principle, benefit principle and marginal benefit principle.

Baj etic (2012) states that the pricing of water services is affected by costs, types and methods of providing services, the demand for water, public and customer relations. The structure of the cost of water services in this case is formed on the basis of (1) costs, (2) values, and (3) price from the competition.

Pricing irrigation water in the Member States is considered in detail in the work Berbel, et al. (2007). The implementation of the WFD (Water Framework Directive) is thoroughly described in

WATECO Guide (2003). EU countries have developed the concept of complete coverage of the cost of water (full cost recovery).

Mesa-Jurado et al. (2008) have presented in their investigation the valuation of water under different scenarios. Aggregated basin value is given as the function relating water value (price) and irrigation consumption.

The economic value of water in agriculture has been considered in research Kasnakoglu & Cakmak (2007). They developed a model in which the economic value of water is determined according to several limitations: different soil types, regions, crops, irrigated land. The model is applied to Anatolia Project in Turkey.

In his paper, Monteiro (2010) systematizes the method of determining the price of water. The marginal method in this case had the highest efficiency in the application. The implementation of each method depended on the way of water supply and demand.

Looking at the present situation and development of water infrastructure for irrigation purposes in the Republic of Serbia, study in this case is focused on the selection of the appropriate method for calculating the cost of irrigation water to suit our economic system and measures of agricultural and water policy.

### **Material and methods**

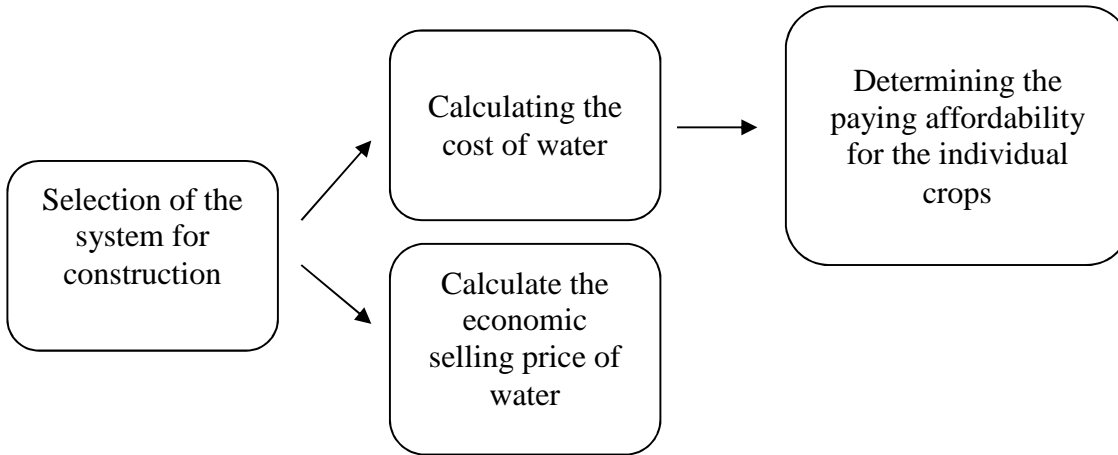
The methods that would be the most appropriate in this case for use in irrigation systems are binomial tariffication water system to the users and the economic cost of water in the long term management of the system (ILRIC).

When binomial tariff is used, irrigation costs consist of two parts: 1) a fixed costs (amortization, maintenance, gross wages of full-time employees, insurance, interest) and 2) variable costs (energy, seasonal labor, maintenance). Under this method, the fixed costs are distributed on the entire irrigated area (din/ha, €/ha). On the other hand, the variable costs are allocated by the level of the water consumption ( $\text{m}^3/\text{ha} * \text{din}/\text{m}^3$ ,  $\text{m}^3/\text{ha} * \text{€}/\text{m}^3$ ). In this way, the crops that consume more water will have higher costs per unit of irrigated surface. Principle based on customer's willingness to pay can be used so that crops such as wheat, forage crops pay a lower cost of irrigation in relation to intensive crops (fruits, vegetables).

From the socio-economic point of view, irrespectively of the conditions of investment financing in irrigation projects (regional or local), calculating the economic cost of irrigation water that will survive in the long term exploitation of the system can be made using the method ILRIC (long run incremental economic prices method). On these terms, economic cost of  $1\text{m}^3$  of water on the same regional sub-irrigation system had been calculated using the given method which is adapted to the characteristics of the system. The basis for establishing selling prices is economic costs calculated for different discount rates. This price should ensure that the total revenue from the sale of water system to users (farms), averaged throughout the century, covers the cost of capital (investment and replacement) and operating costs (maintenance, insurance, labor, energy, material costs). In addition, the accumulation of the amount applied discount rates should be achieved (the discount rate in this case ranged from 0-10%).

**Results of Research. Case study**

The procedure applied in this case consists of:



The system chosen was a regional subsystem "Srem" of 1700 ha and the water source is the River Danube. Following the proposed technical solution of a subsystem using the binomial tariffs and ILRIC method the price of water was calculated (Tables 1 and 2). In the binomial tariff, financing conditions were included as well.

Table 1. Total costs of exploitation-an example of regional subsystem „Srem“

No.	Costs	Amount, €		
		interest, 0%	interest, 3%	after repayment
<b>I</b>	<b>Fixed costs</b>	742883	946943	428162
1	Amortization	237415	237415	237415
2	Maintenance	145147	145147	145147
3	Labor power	45600	45600	45600
4	Interest on credits	0	154368	0
5	Uncovered part of annuity	314721	364413	0
<b>II</b>	<b>Variable costs</b>	118440	118440	118440
6	Energy	109440	109440	109440
7	Material costs	9000	9000	9000
<b>III</b>	<b>Overheads</b>	86132.3	106538.3	54660.2
<b>IV</b>	<b>Total costs</b>	947455.3	1171921.3	601262.2
	<b>Unit variable costs, €m<sup>3</sup></b>	0.0348	0.0348	0.0348
	<b>Unit total costs, €m<sup>3</sup></b>	0.2786	0.34468	0.17684
	<b>Unit total costs without amortization, €m<sup>3</sup></b>	0.2786	0.3446	0.1070
	<b>Area of subsystems, ha</b>	1700	1700	1700
	<b>Planned annual consumption, m<sup>3</sup></b>	3400000	3400000	3400000
	<b>Irrigation costs, €ha</b>	557.32	689.37	353.68
	<b>Unit fixed costs, €ha</b>	436.99	557.03	251.86

Table 2. Selling price of water for regional subsystem „Srem“

Years	Water quantity,m <sup>3</sup>	Nominal value						Total, €	Total with investment, €
		Investment, €	Replacement, €	Maintenance and insurance, €	Labor power, €	Energy, €	Other costs, €		
1		11,199,244							11,199,244
2	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
3	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
4	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
5	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
6	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
7	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
8	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
9	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
10	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
11	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
12	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
13	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
14	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
15	3,400,000		2,917,200	242,388	45,600	109,440	87,970	485,398	3,402,598
16	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
17	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
18	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
19	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
20	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
21	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
22	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
23	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
24	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
25	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
26	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
27	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
28	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
29	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
30	3,400,000			242,388	45,600	109,440	87,970	485,398	485,398
Total	98,600,000	11,199,244	2,917,200	7,029,252	1,322,400	3,173,760	2,551,130	14,076,542	28,192,986
Total (%)		40	10	25	5	11	9	50	100
PW by rate 0%	98,600,000	11,199,244	2,917,200	7,029,252	1,322,400	3,173,760	2,551,130	14,076,542	28,192,986
Economic price		0.11	0.03	0.07	0.01	0.03	0.026	0.14	0.29
PW by rate 6%	43,592,879	10,565,325	1,217,246	3,107,762	584,657	1,403,178	1,127,902	6,223,499	18,006,069
Economic price		0.24	0.03	0.07	0.01	0.03	0.026	0.14	0.41
PW by rate 8%	35,128,315	10,369,670	919,623	2,504,318	471,133	1,130,718	908,893	5,015,063	16,304,356
Economic price		0.30	0.03	0.07	0.01	0.03	0.026	0.14	0.46
PW by rate 10%	28,960,600	10,181,131	698,354	2,064,618	388,413	932,191	749,313	4,134,535	15,014,020
Economic price		0.35	0.02	0.07	0.01	0.03	0.026	0.14	0.52

Considering the yield that can be achieved under irrigation in this area, as well as the selling prices of selected agricultural products the amount of product (t/ha) which should cover the cost of water on the water intake was calculated (Table 3). For most of the crops, additional yield to cover the cost of irrigation is not impossible to achieve.

In addition to these costs, the budget should be expanded to cover the complete cost of irrigation (full cost recovery) including: a) on farm costs, b) resource costs and c) environmental costs.

Table 3. The products need to cover the cost of water intake

No.	Crops	Yield, t/ha	Price, €/ton	Fixed costs, €/ha	Variable costs, €/ha	Costs on hydrant, €/ha	Need, t/ha
1	Wheat	7	170	292.2	31.32	323.52	1.90
2	Maize	12	150	487	69.60	556.60	3.71
3	Sugar beet	65	50	487	83.52	570.52	11.41
4	Soybean	4	350	487	41.76	528.76	1.51
5	Alfalfa	14	120	487	69.60	556.60	4.63
6	Peas	7	300	487	27.84	514.84	1.72
7	Onions	28	200	487	69.60	556.60	2.78
8	Beans	2	1200	487	41.76	528.76	0.44
9	Potatoes	35	160	487	55.68	542.68	3.39
10	Tomato	45	210	487	83.52	570.52	2.72
11	Pepper	35	280	487	83.52	570.52	2.03
12	Cucumber	30	190	487	69.60	556.60	2.92
13	Carrot	30	180	487	69.60	556.60	3.09
14	Apple	25	250	487	83.52	570.52	2.28
15	Pear	20	350	487	83.52	570.52	1.63
16	Plum	25	150	487	76.56	563.56	3.75
17	Peach	25	300	487	83.52	570.52	1.90
18	Apricot	22	320	487	83.52	570.52	1.78
19	Vineyards	12	300	487	62.64	549.64	1.83
20	Double vegetable	20	150	194.8	41.76	236.56	1.57
21	Double forage crops	35	40	194.8	55.68	250.48	6.26

### Conclusion

Pricing irrigation water is always a topical field of research. The price depends on agricultural and water policies of each country and is changeable due to supply and demand for water resources.

The research in this paper is based on the selection of appropriate methods that would be used to determine the price of water. There were two methods (binomial rates and economic sales price of water), which would correspond with the budget price of irrigation water in the Republic of Serbia.

On the example of specific regional subsystem in Srem, Vojvodina, the rates of irrigation water had been calculated based upon selected method.

Also, bearing in mind the principle of payment according to the possibilities of users, costs of irrigation in certain crops were distributed on basis of fixed and variable costs. The required amount of yield for selected crops which needs to be allocated to cover the costs of irrigation on the water intake was also calculated.

The research should be extended to the development of methods for the calculation of resource and environmental costs that are to be included in the price of irrigation water to ensure full cost recovery.

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**USE OF ECOLOGICAL CHARACTERISTIC OF SOIL FOR MAKING THE  
SUITABILITY MAPS FOR GROWTH AND CULTIVATION OF SPECIES OF THE  
GENUS *VACCINIUM* USING GIS TOOLS**

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**Abstract**

Basis for understanding the spatial distribution of specific plant species is in the knowledge of the specific pedoclimatic and orographic demands certain plants.

In this paper authors prepared suitability map for the growth and cultivation of species of the genus *Vaccinium* in the Canton Srednja Bosna using GIS tools. In paper, as the major limitations for growth and cultivation species of the genus *Vaccinium*, it's used pH value of the soil (source: Basic Soil Map of Bosnia and Herzegovina (BiH), 1:50 000), elevation and aspect (source: DTM of BiH). Map as the end result allows the user to get answers to questions related to the cultivation of and growth of species of the genus *Vaccinium* in the Canton Srednja Bosna.

**Keywords:** analysis, GIS, limiting factors, *Vaccinium*

**Introduction**

Plant species, of the genus *Vaccinium*, originating from the eastern part of North America where they were transferred to Europe. A typical representative of the genus *Vaccinium* species is blueberry (*Vaccinium myrtillus* L.) and Northern highbush blueberry (*Vaccinium corymbosum* L.) which are the focus of research to create distribution maps and benefits from the exploitation of natural populations and breeding in Central Bosnia Canton. Blueberries from natural populations (*Vaccinium myrtillus* L.) grow in continental areas in mixed and coniferous forests on acid soil. Optimally grows at an altitude of 800-1200 m above sea level. Often grow in such numbers that fully covers the populated area. Important plant species as growing in poor soil that enriched with humus. From the surface of the soil is much branched subshrub, tall and up to 40 cm. The branches are green and sharp edges. The leaves are finely toothed and leathery, egg-shaped, the edge finely serrated toothed, and in their axillary, from May to June to develop a greenish pink bell-shaped flowers. Individual flowers are placed in the armpit leaf and they are oval shape. In their place in July dark blue berries are maturing that in the upper end ends with circular hollow. Berries are fine pungent odor similar to the smell of fresh wood. Fresh berries are sour - sweet taste, while the dry slightly bitter. The juice berries is dark blue, almost black. Blueberry fruit we collect when they fully mature. They are dried at a temperature of 40 – 50 °C. Its cultivation and increased production gains in importance in recent years thanks to its rich composition, antioxidant, so besides the food, vitamin and cosmetic industry is also interested in this type of healing fruit.

Northern highbush blueberry (*Vaccinium corymbosum* L.) was introduced in the flora of Bosnia and Herzegovina due to import seedlings and start cultivating blueberries. However, both abroad and in our country, began with the breeding of new varieties. They are mostly of American origin and enriched by the so-called. "High shrub" blueberry (which at the peak of the shoot or branch educated offspring by which are extended in height). These types of blueberries do not spread the rhizomes that it makes ordinary blueberries (*Vaccinium myrtillus* L.). These crossbred varieties are characterized by autogamy so it is essential carry out the planting with at least two varieties to increase yield (planting two rows of each variety). In Central Bosnia Canton blueberry

(*Vaccinium myrtillus* L.) and Northern highbush blueberry (*Vaccinium corimbosum* L.) significantly represented in the municipalities: Fojnica, Kiseljak, Kreševo, Travnik, Gornji Vakuf, Donji Vakuf, Jajce and Bugojno. In these areas blueberry has economic importance, given that the export-oriented and highly profitable medicinal plant species. However, natural resources blueberries are not evenly distributed and vary spatially. For this reason it is spatially oriented information base for management of natural resources, in these exact research resource blueberries from natural populations.

The technology intended for the management of spatially oriented data, is GIS (geographic information system). Using GIS technology aims to better and more efficient decision-making in creation of natural resource management and protection of resources, above all, forests, biodiversity and land as a significant resource for sustainable development. Practical application of GIS begins with inventarisation habitats, which includes a variety of procedures, which ultimately results in a single list of all the habitats, distribution of certain plant species in a certain area (municipality, canton or wide area). Inventory of habitat is one of the first and initial steps for all future actions and it is implemented by the relevant phytocenological principles, a detailed list of the distribution and abundance. Often underestimate what it takes to set up a GIS. Unlike office software such as Word, which simply allows you to get started, GIS software is a "semi-prepared". It is necessary to complete and fill out the data could be used. This is because each GIS must be adapted to the specific needs that will be used. Therefore it is necessary to program which data should be able to relate. Then the data is collected and entered into the system. Later this data is updated regularly. Depending on the purpose of GIS, it requires skills and very often, a lot of time. Implementation of GIS should be viewed as a project with clearly defined goals and stages. It is a complex process that can be accomplished only by the principle - step by step. Implementations of GIS include not only the technical aspects. As most often leads to changes in operating procedures, usually requires adjustment of the organizational structure. In certain cases are needed to adapt the legal framework. Finally, the successful implementation of a GIS is often necessary to change the mindset. Any person who makes decisions and who wants start the project of GIS implementation must be aware of the social, organizational and legal changes that accompany the introduction of new technology and, therefore, positively support the process of professional change management.

This paper addresses all the necessary aspects of the application of GIS tools in the case of ordinary blueberries (*Vaccinium myrtillus* L.) and Northern highbush blueberry (*Vaccinium corimbosum* L.).

The main objective of this paper is to determine the habitat distribution of blueberries in Central Bosnia Canton. The next step is to create a map of distribution using GIS tools. Map aims to answer the question "where a habitat for blueberries is and what are the conditions for its distribution and abundance? "i.e. what is the spatial distribution of habitats, which are soil management and pedological aspects (primarily pH, altitude etc.) typical for the habitat of blueberries in Central Bosnia Canton? In the map data is inserted from the field work about distribution of blueberries, then marking the protected areas or specific characteristics, monitoring changes in the habitat over time. Regardless of the actions and habitat mapping techniques and methods of Remote Sensing (preparation and analysis of satellite imagery and aerial photogrammetry) it's always necessary to carry out the field research, and therefore this is an on-going process. For starting position abundance and distribution of blueberries and sustainability of this medicinal plant species, the goal is to establish exactly the maximum annual allowable amount of exploitation "quota" to be the Forest Management Company of Central Bosnia Canton to respect and implement in the general production practice.

## Materials and methods

GIS analysis was performed in the software package ArcGIS Desktop, ESRI. Most analyses involving geomorphology, namely relief facilities require the use of digital terrain model (DTM). DTM represents a set of points on the surface of the Earth, whose spatial coordinates x, y, z are stored on the carrier suitable for further computer processing. As such it is defined as a mathematical model of the Earth's physical surface presented discrete sizes, stored on data storage media. To create a DTM have been used vectorized contour lines and elevation points from 1:25,000 scale topographic maps. In this paper we prepare suitability map for the growth and cultivation of ordinary blueberries (*Vaccinium myrtillus L.*) and Northern highbush blueberry (*Vaccinium corimbosum L.*) in Central Bosnia Canton using GIS tools. In this paper as limiting factors for the growth and cultivation of blueberries we considered pH value of the land (source: Basic Soil Map of Bosnia and Herzegovina, 1:50 000) and elevation (source: DTM BiH).

Habitat mapping was carried out as follows:

- *Geographically (the study of specific areas of distribution of ordinary blueberries and Northern highbush blueberry)*
- *Thematically (mapped are only certain types of habitat - habitat blueberries).*
- *Geographical and thematic (mapped are only certain types of habitats in a particular geographic limited area).*

Map the habitats in nature meant identifying and locating habitats. Periodic repetition of the habitat mapping was done for reasons of monitoring changes in habitat.

Before access to mapping and monitoring abundance and distribution of blueberries determine the important parameters:

- Revealed changes that are occurring on the site, in order to be able to assess the level of vulnerability of blueberries (unsustainable exploitation, wrong tools for exploitation, the exploitation and so on).
- Specifies the methods to ensure the value of the data, how to apply the method of habitat mapping.
- Conducted a consultative process (including the names of evaluators to assess the distribution and abundance of blueberries).

## Results and discussion

Processes endangering certain plant species, therefore blueberries, natural and anthropogenic factors, and induced changes in the population, are mainly continuous. This continuous process of crossing certain economically important medicinal plants in endangered, rare, risky or protected category in the wider manufacturing practices are seen as more or less empirically. Sustainability of blueberries from natural populations and determining the maximum annual amount allowed for exploitation "quota" was carried out precisely in the two-year studies (2011 and 2012) in the Central Bosnia Canton.

Based on the objective estimates prevalence and abundance of blueberries from natural populations there are maximum annual amount allowed collecting "quotas" in the municipalities of the Central Bosnia Canton. The definition of "quota" estimate is calculated based on net square and fresh biomass in tons (t). In determining the "quota", acknowledge the fact that the specified maximum annual amount will not threaten the viability of the species.

Table 1. Allowable annual collection "quota" blueberry *V. myrtillus*

Blueberry / Municipality	Bugojno, G. Vakuf,	Busova a, Vitez,	Jajce, Dobreti ,	Kreševo Kiseljak N. Travnik	Travnik
Allowable annual exploitation "quota"	tons / quotas	tons / quotas	tons / quotas	tons / quotas	tons / quotas
Blueberry ( <i>V. myrtillus</i> )2011	60	0	20	10	40
Blueberry ( <i>V. myrtillus</i> ) 2012	40	0	15	8	30

Of the total area of Central Bosnia Canton 318 930 ha on area suitable for growing blueberries, taking into account as a limiting criteria elevation we come to an area of 120 317.65 hectares or 37.73%. (Figure 1. and Table 2.).

Table 2. Suitability of growing blueberries in relation to elevation

Altitudes	Suitability for growing blueberries	Area (ha)	Percentage
from 800 to 1000 m	Suitable for growing blueberries	120317,65	37,73
below 800 and over 1200 m	Unsuitable for growing blueberries	198612,35	62,27
<b>Total</b>		<b>318930,00</b>	<b>100,00</b>

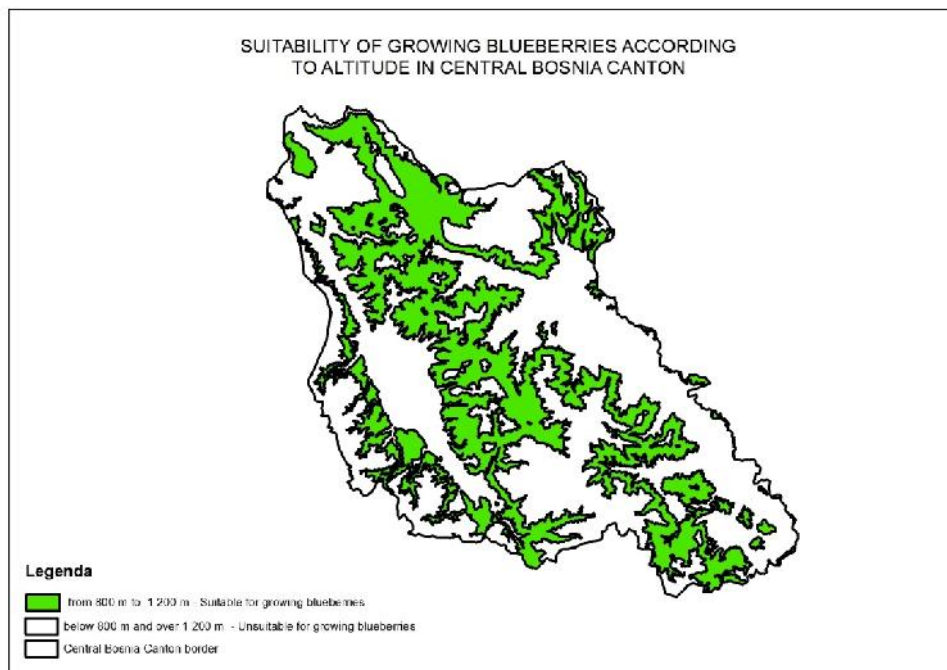


Figure 1. Suitability of growing blueberries according to altitude in Central Bosnia Canton

Taking into account the pH value, as another limiting factor for growing blueberries we come to data on benefits of growing blueberries in an area of 147 136 ha, or 46.13% of the total area of Central Bosnia Canton (Tables 3 and 4 and Figure 2).As a source for obtaining information on soil reaction in H<sub>2</sub>O used data from the "Basic Soil Map of Bosnia and Herzegovina of 1:50 000."

Table 3. Suitability of growing blueberries in relation to pH

<i>Soil reaction in H2O</i>	<i>Suitability for growing blueberries</i>	<i>Area (ha)</i>	<i>Percentage</i>
Areas without soil		624,00	0,20
<b>pH less than 4.5</b>	<b>Suitable for growing blueberries</b>	<b>68413,00</b>	<b>21,45</b>
<b>pH 4.5 to 5.5</b>	<b>Suitable for growing blueberries</b>	<b>78723,00</b>	<b>24,68</b>
pH 5,5 to 6,5	Unsuitable for growing blueberries	25778,00	8,08
pH 6,5 to 7,2	Unsuitable for growing blueberries	112436,00	35,25
pH higher than 7.2	Unsuitable for growing blueberries	32956,00	10,33
<b>Total</b>		<b>318930,00</b>	<b>100,00</b>

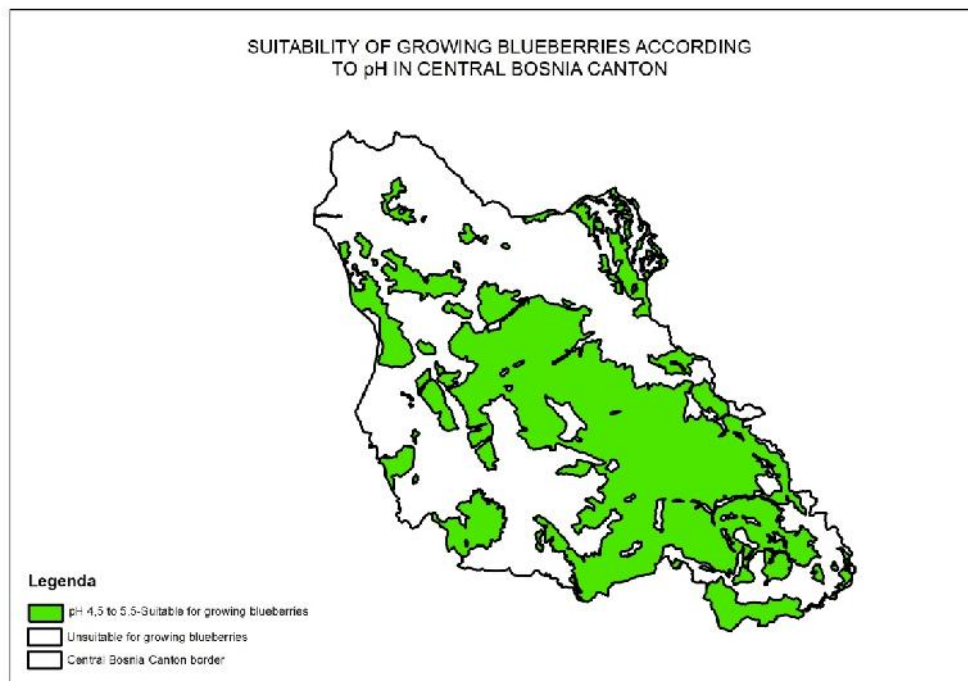


Figure 2. Suitability of growing blueberries according to pH in Central Bosnia Canton

Table 4. Suitability of growing blueberries in relation to pH

<i>Reaction suitable for growing blueberries</i>	<i>Area (ha)</i>	<i>The percentages of the total area of Canton</i>
pH less than 4.5	68413,00	21,45
pH 4,5 to 5,5	78723,00	24,68
<b>Total</b>	<b>147136,00</b>	<b>46,13</b>

By applying tools for the analysis of spatial data available in ArcGIS, and with respect to mentioned limiting factors (altitude and the pH value of the soil), we come the conclusion that the

from total area of Central Bosnia Canton (318,930 ha) only 56.450,00 hectares or 17.70 % of the area suitable for the growth and cultivation of blueberries (Figure 3).

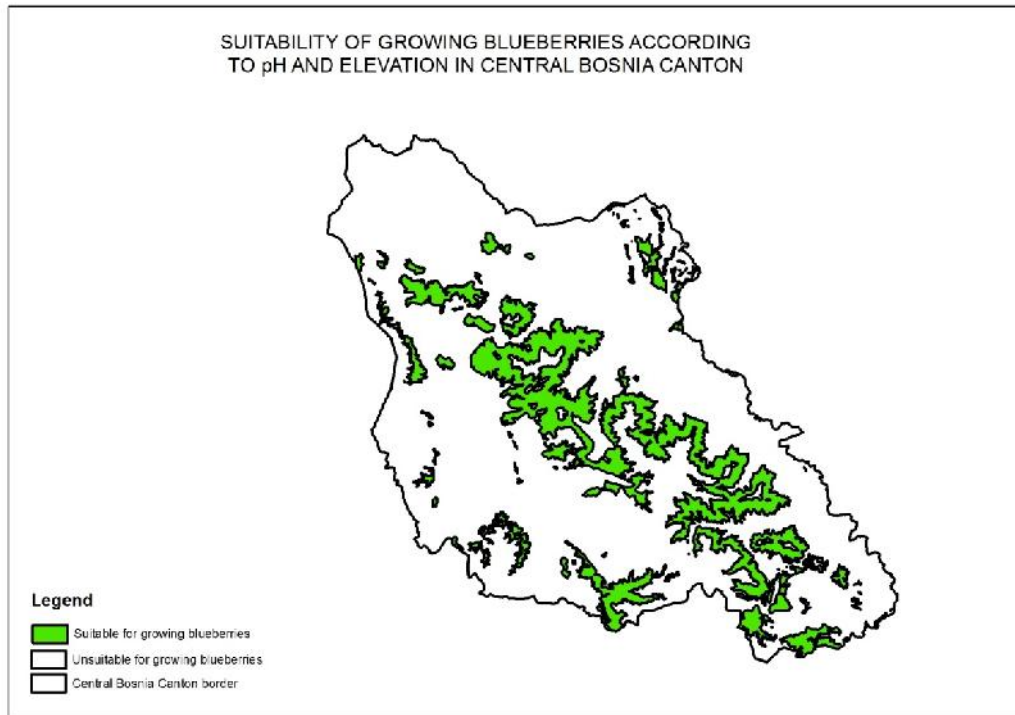


Figure 3. Suitability of growing blueberries according to pH and elevation

Using the method described in this paper Forest Management Company which is responsible for the sustainable management of forest resources in the area of Central Bosnia Canton can make plans for the exploitation of non-wood forest products. From the aspect of agriculture by using this method it is possible to adopt programs and policies for establishing new plantations of Northern highbush blueberry (*Vaccinium corimbosum L.*) with making geographic and alphanumeric databases on individual plantations in a certain area and cadastral parcels. For blueberries from natural populations (*Vaccinium myrtillus*) we highlight areas of distribution according to pH and altitude. The precise study determined the allowed annual exploitation (collection) "quota" to the sustainability of the species.

### Conclusion

Based on the two-year study of distribution blueberries (*Vaccinium myrtillis L.*) and the possibility of growing Northern highbush blueberry (*Vaccinium corimbosum L.*), determining the maximum annual amount of exploitation and a number of other issues for the overall MAP (Medicinal and Aromatic Plants ) sector, it is necessary to test and enhance the necessary key issues that need to be constantly upgraded (list - inventory, quota system, mapping, training of collectors, the introduction of the best sustainable practices and certification, local brands, etc.).

In the context of exact research can be derived the following conclusions:

- Using the example of blueberries to make mapping and analysis of other habitats of medicinal plants in order to determine the number and distribution, and certainly changes that occur on the site in order to be able to assess the degree of endangerment.
- To use methods for habitat mapping based on maps and aerial images, habitat mapping using grids of point, or by working on permanent plots with suitable equipment.

- For mapping and monitoring of habitat to use digital tools: GPS devices, Internet, GIS, etc. in order to geocode locations, capture additional data, their storage, and analysis of certain plant species, total MAP sector (use of Medicinal and Aromatic Plants).
- According to our research, in the case of blueberries (*Vaccinium myrtillis* L.) and the possibility of growing Northern highbush blueberry (*Vaccinium corimbosum* L.), most effective was digitalization of management of MAP sector, due to the large number and size of existing data it is necessary to use GIS as a tool to support decision-making process and can contribute significantly to the design of administrative and management procedures that are more efficient, transparent and easy to use in a number of activities, therefore in the MAP sector.
- In practical terms, the use of GIS technology contributes to the quality of decision-making with the new capabilities of data analysis, examining the data faster, more efficient communication with municipalities and cantons, handling and analysis of a large number of data, increased efficiency in the public procedures.
- Determining the maximum annual amount of exploitation "quota" in the case of wild blueberries can serve for Forest Management Company of the Central Bosnia Canton (as a model can be unified and to other areas), permitting certain subjects on permitted amounts of collection and inspected to support the sustainability of resources.

### Literature

GIS by ESRI Getting Started with ArcGIS™

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**DYNAMICS OF THE WATER CONTENT IN THE SOIL DURING THE PERIODS  
WITHOUT PRECIPITATION IN THE BOCEGAJ SUBCATCHMENT IN 2009 AND 2010**

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**Abstract**

The decrease trend of volumetric soil water content was observed during the dry season of 30 days with the rainfall up to 3 mm in 2009 and 2010; 8.9.-8.10.2009 (30 days) and 23.6.-22.7.2010 (29 days) in Bocegaj subcatchment, Slovakia. The dynamics vary between the observation places and depth. The highest decrease was up to 300 mm depth, the lowest in depth 1000 mm. The differences were mainly up to 10 percent of volume (average 4.3 % vol.) in 2009 and over 15 percent of volume (average 13.3 % vol.) in 2010. Higher temperature, soil type and texture, and different crops influenced the recession of the soil moisture in 2010.

**Key words:** soil drought, volumetric soil water content, dynamics of soil water content

**Introduction**

The landscape structure is being changed, as well as the processes connected to the landscape. One of these processes is the water cycle. Knowledge of soil water content and its dynamic is significant, especially for farmers. They might be able to react in the time to avoid the water stress of plants by additional irrigation.

Soil moisture is an important component in the atmospheric water cycle, both on a small agricultural scale and in large-scale modeling of land - atmosphere interaction (WMO, 2008). Soil moisture should be measured at a location most representative of a watershed when climatic data or vegetative conditions indicate a likelihood of actual conditions (NRCS).

Rainfall and snowmelt are natural sources of soil water and are normally greatly reduced during drought. Slope shape, gradient, and soil surface roughness will affect soil water content since surface or subsurface run-on from adjacent upslope sites can add to the soil moisture, while surface runoff can remove water from a site. Evaporation, plant transpiration, and deep percolation beyond rooting depth are other factors that deplete soil moisture (NRCS).

Drought is a normal, recurrent feature of climate, although often erroneously considered an unexpected and extraordinary event. It occurs in virtually all climatic zones, but its characteristics vary significantly from one region to another. Drought is a temporary aberration within the natural variability and can be considered an insidious hazard of nature; it differs from aridity which is a long-term, average feature of climate (EUWI, 2007). It is often difficult to know when a drought begins. Likewise, it is also difficult to determine when a drought is over and according to what criteria this determination should be made. Intensity refers to the degree of the precipitation shortfall and/or the severity of impacts associated with the shortfall (WMO, 2006).

Droughts are commonly classified by type as meteorological, agricultural, hydrological and socioeconomic. *Meteorological drought* is usually defined by a precipitation deficiency threshold over a predetermined period of time. *Agricultural drought* is defined more commonly by the availability of soil water to support crop and forage growth than by the departure of normal precipitation over some specified period of time. *Hydrological drought* is even further removed from the precipitation deficiency since it is normally defined by the departure of surface and subsurface water supplies from some average condition at various points in time. *Socio-economic*

*drought* differs markedly from the other types of drought because it reflects the relationship between the supply and demand for some commodity or economic good, such as water, livestock forage or hydroelectric power that is dependent on precipitation (WMO, 2006; EUWI, 2007).

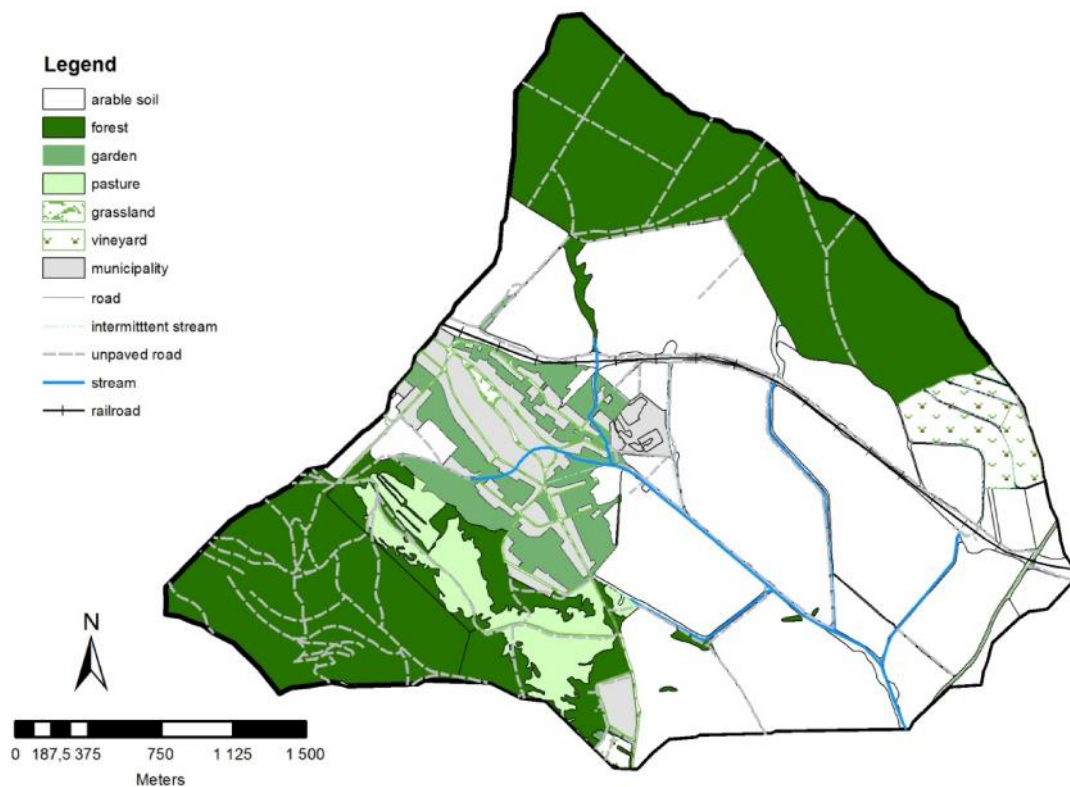
The aim of a paper is to show the dynamic of the soil moisture in an arable soil up to 1 m depth in the subcatchment Bocegaj during the period without precipitation in the years 2009 and 2010.

### Materials and methods

The area of interest is a subcatchment of the Bocegaj stream with an area of 9.75 square kilometres. It is situated in Southeast Slovakia, approximately 10 kilometres northeast of Nitra. Daily average air temperature is 9.8 °C (mean value for years 1961-1990) in the Nitra region, and total yearly precipitation is 539 millimetres (mean value for years 1961-1990). Soil types are mainly represented by Haplic Luvisol (52 percent of arable soil), Cambisols (37.5 percent) and Fluvisols (10.5 percent). Soil texture groups are represented by loam top soil and clay loam subsoil (62.1 percent of arable soil), clay loam (15.3 percent), loam (7.5 percent), and sandy loam soils (15.1 percent). According to information from hydrogeologists, the relatively confined aquifer is quite variable under the terrain, at a range of approximately between 2 and 12 metres. The same situation exists with ground water level. The area of interest is detailed present in work Kaletova (2011).

The soil moisture by volume was monitored from the 8<sup>th</sup> September 2009 to the 24<sup>th</sup> November 2010 at a depth of 1 meter in the 5 observation places. The soil moisture was measured by the Profile Probe PR2/6 (Delta-T Device Ltd.) with connection to the Moisture Meter HH2 (Delta-T Device Ltd.). The Profile Probe PR2/6 uses frequency domain reflectometry to measure volumetric soil moisture. The observation places were constructed on the edge of the arable soil cross the area to be protected before the damage by farmers.

Two seasons with the total rainfall up to 3 mm were chosen; 8.9.-8.10.2009 (30 days) and 23.6.-22.7.2010 (29 days). We assumed that rainfalls up to 3 mm were intercepted on the vegetation. The measurements were done every fortnight.



## Results and discussion

The values of the volumetric soil water content in % of volume is shown on the figure 2 in depths 100, 200, 300, 400, 600 and 1000 mm. The figure shows only the period without precipitations up to 3 mm. The figure 3 shows differences in % of volume between minimal and maximal value of volumetric soil water content during the period.

There is visible decrease trend of the soil moisture during the period without rainfall up to 3 mm in both years. Higher decreases were in the 2010. The main point is that on the first day of the observation the soil water content was almost equal to field capacity. There was enough soil water to evaporate, and crops were in the growth (tab. 1). Also, the higher temperature, soil type and texture, and different crops influenced the recession of the soil moisture in 2010.

Table 1 Crops grown around the observation places

Place	2009	2010
1	after the tillage; <i>Brassica napus</i> was seeded	<i>Brassica napus</i>
2	after the tillage; <i>Brassica napus</i> was seeded	<i>Brassica napus</i>
3	after the tillage; <i>Triticum aestivum</i> was seeded	<i>Triticum aestivum</i>
4	<i>Medicago sativa</i>	<i>Medicago sativa</i>
5	after the tillage	<i>Cucurbita pepo</i>

The dynamics vary between the observation places and depth (fig. 2). The highest decrease was up to 300 mm depth, the lowest in depth 1000 mm. In the literature (e.g. Dub, 1963) it is written that by the influence of evapotranspiration, arable soil during the dry season is dried up only in the upper layer up to a depth of 150 mm and slightly less in the layer from 150 to 250 mm, the deeper layers dry up very little. The differences were mainly up to 10 percent of volume (average 4.3 % vol.) in 2009. In 2010 the differences were over 15 percent of volume (average 13.3 % vol.) (fig. 3).

The volumetric soil water content descended below the wilting point at the end of observed period in the soil depth of 100 mm in all places. Mainly in 2009 the soil moisture was lower than the wilting point. The wilting point was defined according to soil type and texture (range 17 – 23 %).

The decrease of volumetric soil water content in lower depths of the profiles was made by the capillary raise of water into upper soil layers, water consumed by plants, and percolation of the soil water into deeper layers. The ground water level was not observed at a depth of 2 m, therefore the influence on soil moisture by capillary raised ground water is not assumed in these observation places.

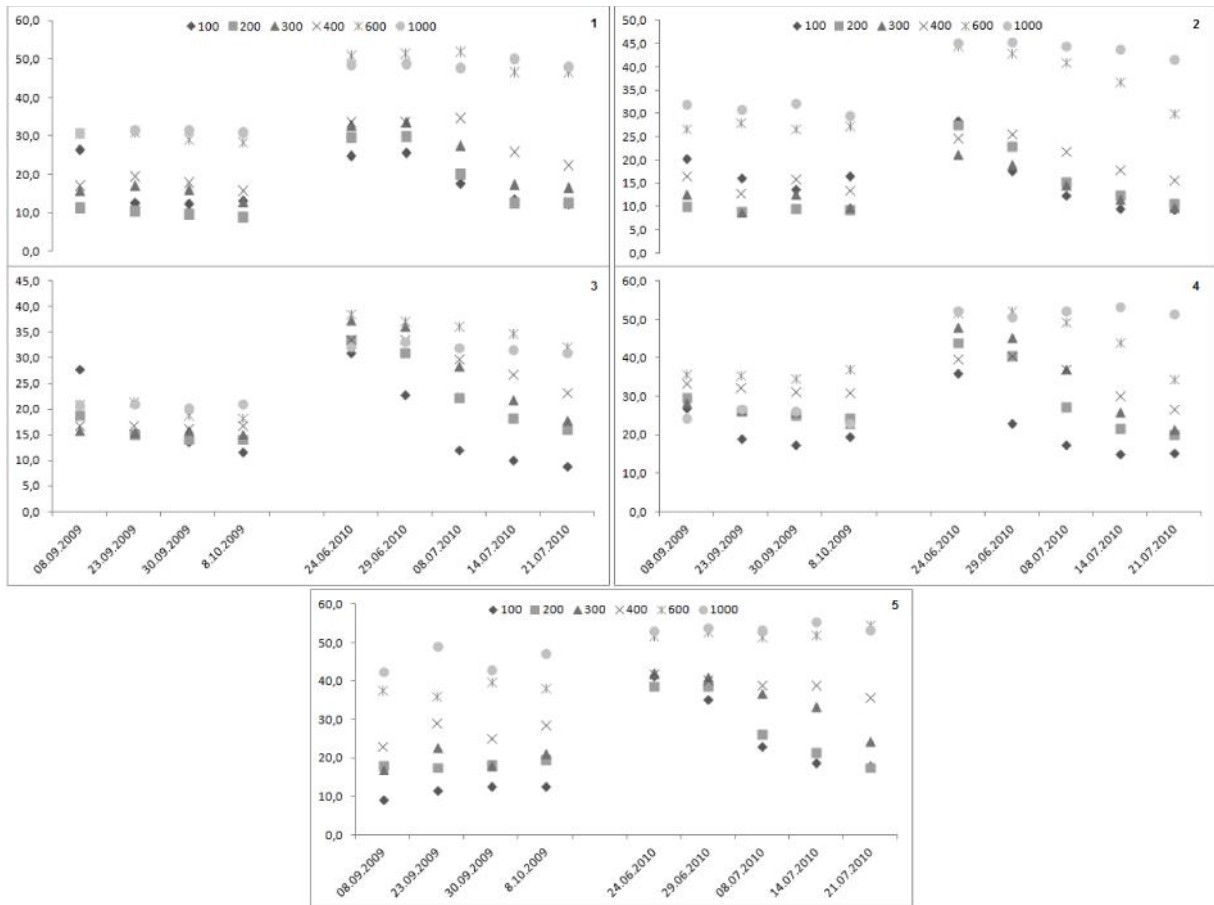


Figure 2 Dynamics of volumetric soil water content (in % vol.) in different depth during the periods without the precipitation

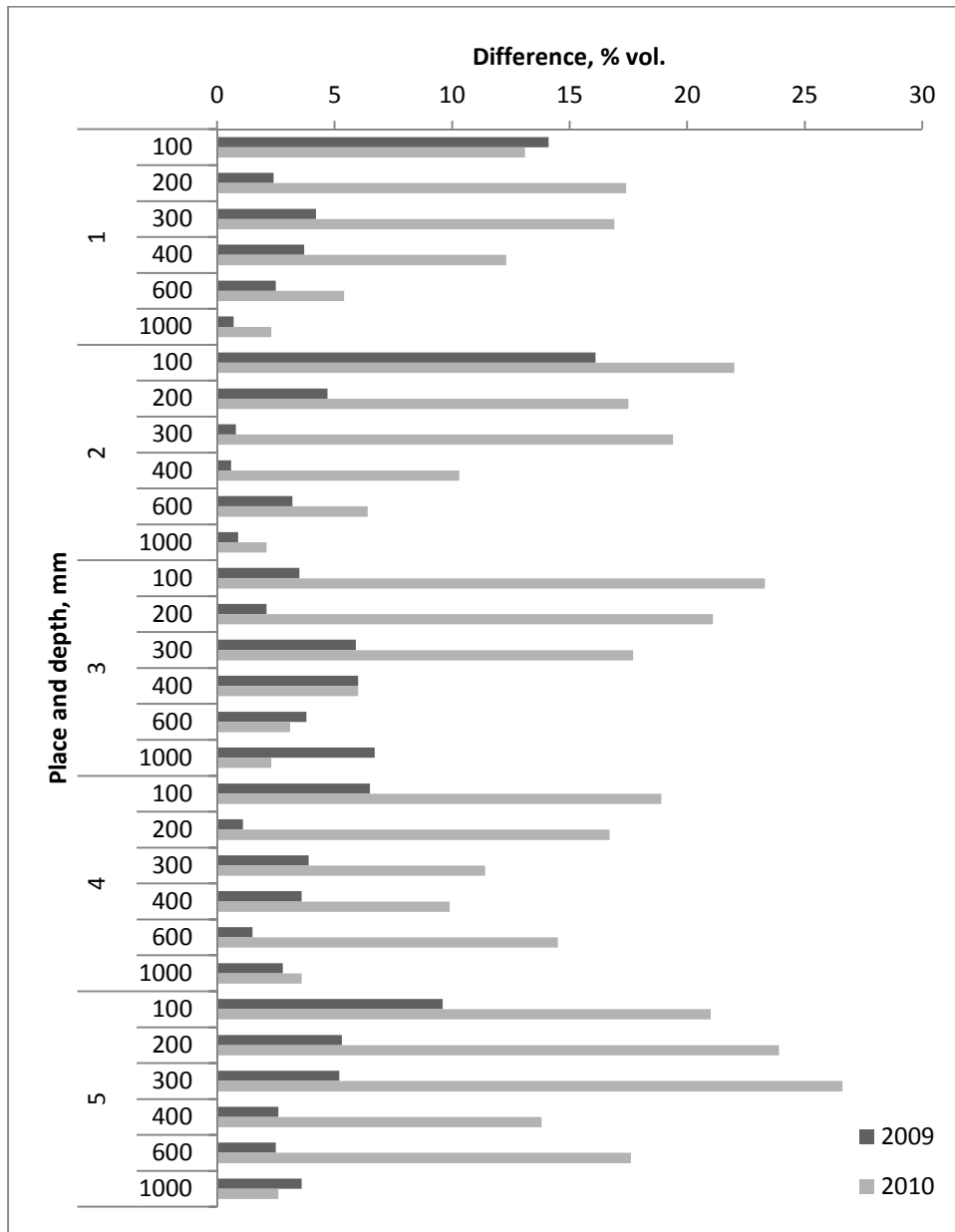


Figure 3 Differences of the soil moisture in different depth and place in the 2009 and 2010

### Conclusion

The decrease trend of volumetric soil water content was observed during the dry season of 30 days with the rainfall up to 3 mm in 2009 and 2010. The main influence on the decrease had the high air temperature, crops and soil type and texture. The higher decrease was in 2010 because of the crops in the soil and higher air temperature. There is evidently visible decrease of the soil moisture in the season without rainfall (approximately 2 volume percent per week in 2009).

Knowing the amount and dynamics of soil water content we may predict and react in time to the current, especially climatic, conditions of the environment. Farmers may determine the date and amount of additional irrigation, or apply other agrotechnical arrangements; water managers may forecast floods and so on.

The landscape, its structure, and its cover are dramatically changed in the agricultural used part during the year and mainly due to the impact of water cycle on evapotranspiration. Repudiation

of the landscape like the part of cycle is a mistake of majority of models, and so results on the differences between simulated and measured values.

### **Acknowledgement**

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## **THE VIEW OF AGRICULTURAL PRODUCERS ON THE LOSS OF AGRICULTURAL LAND IN THE PROCESS OF HYDROPOWER PLANT CONSTRUCTION IN SLOVENIA**

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### **Abstract**

On the one hand the demands for energy are rising, and on the other hand agricultural production and food self-sufficiency have been emphasised. In our study we analysed the views of agricultural producers on the loss of productive agricultural land due to the Brežice and Mokrice hydropower plant (HPP) construction on the lower Sava river in Slovenia and eastern highway construction within HPP Mokrice construction. The study included socio-economic analysis (semi-structured interviews with farmers and land owners) and spatial analysis of the available natural resources in the study area, using geographic information system (GIS). In total 188 questionnaires were analysed, 51 (Brežice), 102 (Mokrice), and 35 (eastern highway-HPP Mokrice). Socio-economic analysis revealed that agricultural producers have low interest in protecting their agricultural land. Based on the available information, we expect that the combination of purchase and replacement of agricultural land will allow those farms for which farming is an important source of revenue in the study area to focus in more development-oriented farming in the future. Therefore the development of appropriate agricultural and rural development programs is necessary.

**Keywords:** Agricultural land management, GIS analysis, hydropower plant construction, food self-sufficiency, natural resource protection.

### **Introduction**

Usefulness of geographic information systems is perceived in many scientific fields, both in natural and social sciences. In general, the use of geographic information system using satellite imagery and aerial photography is as well widely represented in agriculture. This is evidenced by numerous studies from the agricultural field, such as studies in the research of precision farming (Zhang et al., 1999), determining the suitability of soil for agricultural production (Kalagirou, 2002), evaluation of agricultural conservation policies on the farmer's practices (Lant et al., 2005) and evaluation of biomass potential from energy crops at the regional scale (Fiorese & Guariso, 2010). Combining the knowledge of geographic information system with other scientific disciplines is recently gaining in importance. One of the reasons is that spatial representation allows rapid display of land use information to each land parcel precisely, provided that spatial data on land use and land parcels is of good quality. Spatial research in the field of agriculture is also important starting point in the environmental impact assessment studies and evaluation of various environmental interventions on the loss of best productive agricultural land.

### **Materials and methods**

#### **Spatial and statistical data**

For the purposes of spatial analysis various geospatial data needed to be acquired from different sources, such as the database of agricultural land use per individual agricultural holding from Slovenian ministry of agriculture known as the database of Graphical units of agricultural parcels (GERK database), administrative municipality borders from Slovenian ministry of infrastructure

and spatial planning and official borders of the area of permanent land occupation due to the construction of hydropower plant (IBE d.d.-engineering and consulting company) and eastern highway (PNZ d.o.o.-consulting and designing company). Collected data of farm subsidy applications for agri-environmental measures in the year 2007 acquired from the Slovenian agency for agricultural markets and rural development (Farm agency) was used in the case of the detailed agricultural analysis, such as the number of active agricultural households (those receiving subsidies in the agri-environmental payment programme), the size of agricultural parcels, the type of agricultural plant sown on the parcels etc. Agricultural holdings farming land within the area of permanent land occupation were identified from GERK database using ArcMap tools. Agricultural parcels of individual agricultural holding within the case study area were selected with Selection by location tool, meaning that all the parcels, those completely within the boundaries of permanent land occupation due to the construction and those containing only a part of their area inside, were selected and further analysed. In order to extract the non-spatial data of Farm agency for the selected case study, this data needed to be imported into ArcMap and related to the spatial data in attribute table of GERK database using the parcel identification number (GERK PID) from both databases (spatial and non-spatial) as a relation key.

#### Field survey and socio-economic analysis

On the identified agricultural holdings semi-structured interviews were conducted in December 2007 (HPP Brežice), January and February 2008 (HPP Mokrice) and in March 2013 (eastern highway-HPP Mokrice). Interviewing was conducted with the co-operation of agricultural advisors from Agricultural institute of Novo mesto (HPP Brežice and HPP Mokrice) and MSc students from the Agronomy department of Biotechnical faculty in Ljubljana (eastern highway-HPP Mokrice). In total 188 questionnaires were completed and further analysed using Microsoft Excel. The questionnaire was divided in four sections, e.g.: (a) general information about agricultural holding and family members, (b) information about agricultural production, (c) the impact of HPP and highway construction on agricultural holding and (d) information about supplement on-farm activities. In addition selected statistical data of agricultural census 2002 collected by the Statistical office of the Republic of Slovenia was analysed in order to complement the study which was commissioned by the Holding Slovenske elektrarne group (HPP Brežice and HPP Mokrice) and Brežice Municipality (eastern highway-HPP Mokrice).

#### Case study area

In this paper the analysis is focused on the areas of permanent land occupation due to the hydropower plant (HPP) construction on lower Sava river and eastern highway construction within HPP Mokrice construction. In the case of HPP Brežice the area of permanent land occupation is located in two municipalities, Krško and Brežice, whilst in the case of HPP Brežice and eastern highway this area is solely located within the administrative borders of Brežice municipality, bordering Croatia on southeastern part of Slovenia (figure 1). HPP Brežice and HPP Mokrice are the last two in the chain of six HPPs on the lower Sava river in Slovenia with a total average annual production of 281 GWh. Moving towards the more sustainable development in the future the impacts of HPP need to be carefully considered, especially if the loss of best productive agricultural land is involved. The major impact of HPP construction is related with dams and reservoirs, which can permanently occupy bigger areas of actively farmed agricultural land and therefore can have a wider impact on agricultural production and consequently farmers' income.



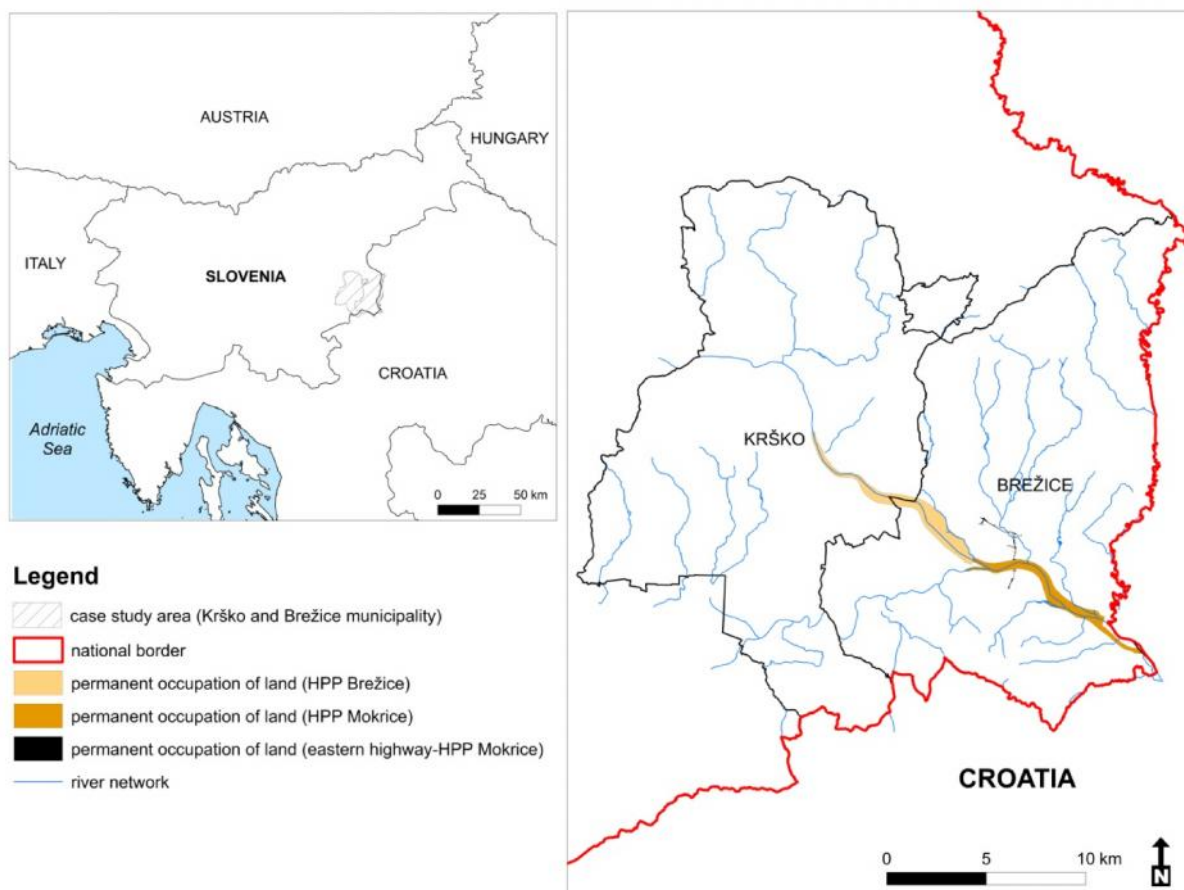


Figure 1. Geographical position of case study area and the area of permanent occupation of land due to the hydropower plant (HPP) and highway construction (Milišević, 2013).

## Results and discussion

### Agricultural holdings and main land parcels characteristics

The spatial analysis of GERK database (2007) revealed that in total 602 land parcels of total 266 agricultural holdings in Krško and Brežice municipality will be permanently affected due to the HPP and highway construction (table 1). It should be noted that all the analysed parcels are actively farmed and farmers or land owners submit claims for agri-environmental measures and claims for less favoured areas on these parcels. Other actively farmed parcels not officially registered in the Register of agricultural holdings cannot be included in the analysis. More detailed analysis revealed that in the study area small land parcels, up to 1 ha, prevail (figure 2). Land parcels bigger than 20 ha are owned by two agricultural companies identified in the area. According to the information of agricultural advisors from Brežice, 90 % and 83 % of land parcels permanently lost due to the construction of HPP Brežice are state owned, in Krško and Brežice municipality respectively. In the case study area of HPP Mokrice construction (in Brežice municipality) the share of state owned land parcels is 25 %. High share of state owned land parcels is related with the farmers' views and future farmers' decisions about farming, that were expressed within the semi-structured interviews.

Table 1. Number of agricultural holdings and land parcels in 2007 according to the GERK database within the permanent land occupation due to the hydropower plant (HPP) and highway construction.

Number of ...	HPP Brežice	HPP Mokrice	Eastern highway (HPP Mokrice)	Total
... agricultural holdings	58	164	44	<b>266</b>
... land parcels	122	402	78	<b>602</b>

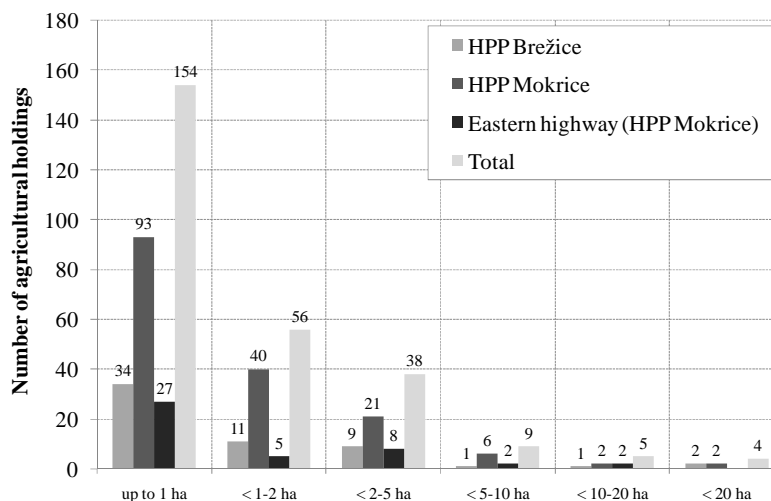


Figure 2. The size of individual agricultural land parcels belonging to one agricultural holding in 2007 according to the GERK database within the permanent land occupation due to the hydropower plant (HPP) and highway construction.

#### Actual land use in 2007

Spatial analysis of land use shows that in 2007 arable land was accounted for the largest area within permanently occupied land due to the HPP and highway construction (table 2). After arable land, the second largest permanently occupied area in the case of HPP Brežice is covered by forest plantations, mainly owned by the HPG Brežice agro-forest company. In the case of HPP Mokrice and eastern highway the second largest land use are meadows and pastures, owned mainly by individual land owners. Bigger land parcels are occupied by arable land and forest plantations, whilst smaller by meadows and pastures (figure 3).

Table 2. Actual land use in 2007 according to the GERK database within the permanent land occupation due to the hydropower plant (HPP) and highway construction.

Actual land use (2007)	HPP Brežice	HPP Mokrice	Eastern highway (HPP Mokrice)
Arable land	264,62	165,84	71,36
Other permanent crops on arable land	9,12	0,00	0,00
Green houses	0,00	0,03	0,00
Intensive orchards	29,75	0,00	0,00
Meadows and pastures	28,13	104,67	11,60
Overgrown areas	2,95	0,75	0,16
Forest plantations	222,36	19,24	0,00
Riparian overgrowth and forest hedges	0,06	0,09	0,00
<b>Total (ha)</b>	<b>556,99</b>	<b>290,62</b>	<b>83,13</b>

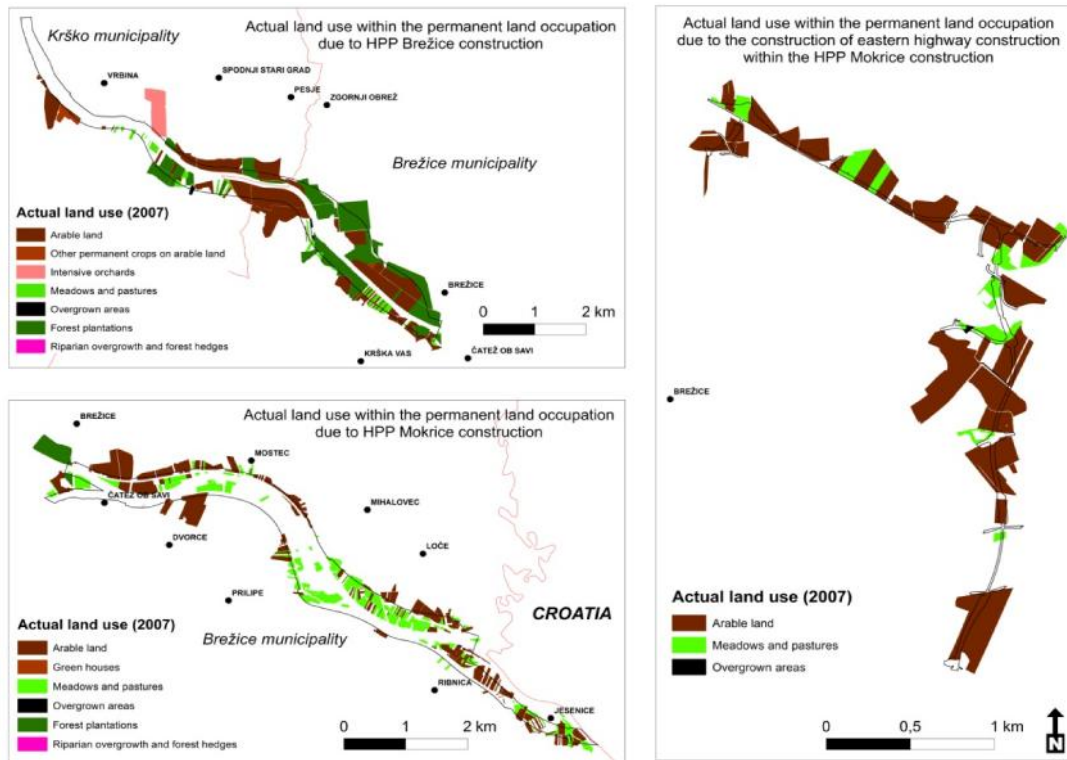


Figure 3. Map of actual land use in 2007 according to the GERK database within the permanent land occupation due to the hydropower plant (HPP) and highway construction (Milićević, 2013).

#### Type of agricultural production in 2007

The relation of non-spatial data from Farm agency and spatial data from Ministry of agriculture reveals that in 2007 farmers were mainly producing wheat and grain maize. This fact is supported by the data of Agriculture census (Statistical office, 2000), where it is stated that agricultural holdings in Krško and Brežice municipality are mainly crop and livestock oriented (figure 4). After wheat and grain maize permanent grassland and clover cover most of the land parcels within the affected area.

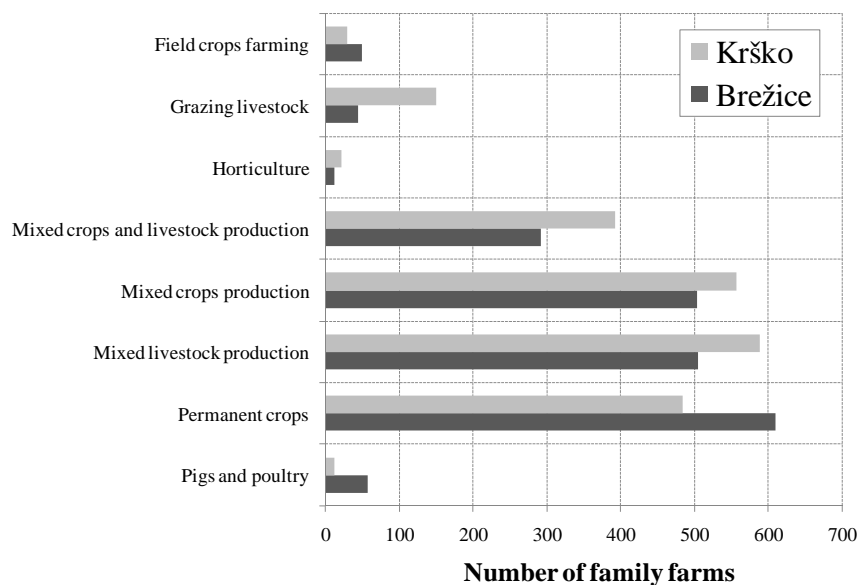


Figure 4. Number of family farms by agriculture type in Krško and Brežice municipality according to the Agricultural census in 2002 (Statistical office, 2002).

## Socio-economic type of agricultural holdings

The results of semi-structured interviews indicate that most of the interviewed agricultural holdings are supplementary (hobby) farms. In the case study area of eastern highway most of the farms are part-time farms (table 3). The definition of each socio-economic farm type was set by Kovačič (1996) and it defines full-time professional farm as generally one in which one of the active members of the core family (farm manager or his spouse or successor to his or her spouse) is not regularly employed outside the farm. Farming therefore represents the main source of income. Part-time farm has at least one active member of the core family, who is fully employed outside the farm and at least one that is fully employed only on the farm. In the case of supplementary farm all active members of the family are regularly employed outside the farm. On the aged farms all household members are older than 64 years.

Table 3. Socio-economic structure of interviewed agricultural holdings.

Socio-economic farm structure	HPP Brežice	HPP Mokrice	Eastern highway (HPP Mokrice)
Full-time professional farm	39	28	21
Part-time farm	12	25	76
Supplementary ("hobby) farm	49	46	0
Aged farm	0	0	3
<b>Total (%)</b>	<b>100</b>	<b>100</b>	<b>100</b>

## Farmer's views on the loss of agricultural land

One of the most important questions to farmers and land owners in semi-structured interviews was the one on their intention on replacement of lost agricultural land due to the planned construction in the case study area. The highest share of farmers and land owners who do not have any intention to replace the lost agricultural land was in the case of HPP Mokrice construction. Farmers and land owners in HPP Mokrice study area do not have strong intention to protect their land, because they mainly own small agricultural plots up to 2 ha which does not represent economically important income for them. The main income for them represents work outside the farm, therefore they do not see any purpose in protecting their land in long term. Apart from that, the other reason is the amount of compensation they will get in return if they will be willing to give away their plot of land within the area of HPP and highway construction. The other group of farmers does not worry about losing their land, because they say they will buy new agricultural land elsewhere and they will continue farming on other location. Our study revealed that farmers' view on protecting agricultural land is strongly related with socio-economic structure of the farm. If farming does not represent the main household income, the lower the intention of replacement of agricultural land is. The third group of farmers would like to get a substitute agricultural land, because they would like to continue farming in the future. A few farmers said that they will increase the intensity of production on the other agricultural land they own elsewhere. Based on the available information, it is expected that the combination of purchase and replacement of agricultural land will allow to those farms which farming is an important source of revenue in the future, that they will be able to continue with farming. The assistance to those affected by the loss, with appropriate development programmes will play an important role. Within that it would be also reasonable to consider about converting from economically less intensive agricultural production (crop and forage production) to more intensive irrigated agriculture (vegetables, strawberries, etc.) and introducing of supplementary activities on the farm, such as (processing of plant and animal products, agro-tourism, social services etc.).

## Conclusions

In this paper the analysis was focused on the areas of permanent land occupation due to the hydropower plant (HPP) construction on lower Sava river and eastern highway construction in Krško and Brežice municipality. The study included socio-economic analysis (semi-structured interviews with farmers and land owners) and spatial analysis of the available natural resources in the study area, using geographic information system (GIS). The spatial analysis of GERK database (2007) revealed that in total 602 land parcels of total 266 agricultural holdings in Krško and Brežice municipality will be permanently affected due to the HPP and highway construction. Spatial analysis of land use showed that in 2007 arable land was accounted for the largest area within permanently occupied land due to the HPP and highway construction, followed by forest plantations, mainly owned by the HPG Brežice agro-forest company, and meadows and pastures, owned mainly by individual land owners. On the affected land parcels farmers mainly produce wheat, grain maize, permanent grassland and clover. This is connected with the type of agricultural production in the area which is more crop and livestock oriented. The results of semi-structured interviews indicates that most of the interviewed agricultural holdings are supplementary (hobby) farms, what is in relation to low intention for replacement of lost agricultural land due to the HPP and highway construction. For them farming does not represent the main household income, therefore their interest of protecting agricultural land is low. For the farmers who are willing to continue with farming it would be reasonable to consider about converting from economically less intensive agricultural production (crop and forage production) to more intensive irrigated agricultural production (vegetables and strawberries). Furthermore introducing of supplementary farm activities (processing plant and animal products, agro-tourism and special social services) need to be considered in the future, for the long term success of farming in the area. To achieve this appropriate agricultural and rural development programmes need to be carefully planned to encourage farmers to continue with appropriate farming practices in the future in order to achieve sufficient food self-supply in the region.

## Acknowledgements

The results of the study presented in this paper were conducted within the environmental impact assessment (EIA) study which was commissioned in 2008 and 2013, by the HSE group and Brežice municipality respectively. The EIA study in this case was done only for the agricultural sector with the emphasis of HPP and highway construction on the loss of productive agricultural land in the case study area.

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**THE LEVEL OF POLLUTION FROM NUTRIENTS AND HUMAN EFFECTS ON  
DURRES BAY**

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**Abstract**

The aim of this study is to assess the pollution of the water in the Bay of Durres from wastewater discharges, including the one generated from livestock units. Monitoring expeditions were carried out in the coast line of Durres Bay. There were appointed 2 sampling points, The first point named Currila is located in the north suburb of the city of Durres and the second point named Plepa in the estuary of the wastewater and livestock water discharges, in the south of Durres city and were analysed physic-chemical indicators in May and August 2011.

The results of the analysis showed evident differences compared to the reference parameters of EU for the bathing water quality. Specifically *Streptococcus Faecalis* resulted  $3.1 \times 10^3$  and  $9.3 \times 10^3$  MPN/100 ml water (May/August) in the Currila point, versus  $3.5 \times 10^4$  and  $9.8 \times 10^6$  MPN/100 ml water (May/August) in the estuary of the wastewater discharges (Plepa point), while the reference parameters of EU for the bathing water quality is  $2 \times 10^3$  MPN/100 ml water.

Specifically *Escherichia coli* resulted  $1.5 \times 10^3$  and  $3.4 \times 10^3$  MPN/100 ml water (May/August) in the Currila point, versus  $2.3 \times 10^5$  and  $9.3 \times 10^6$  MPN/100 ml water (May/August) in the estuary of the wastewater discharges (Plepa point), while the reference parameters of EU for the bathing water quality is  $1 \times 10^4$  MPN/100 ml water. As observed, among others, the seasonal and livestock units effects are evident. It can be concluded that, to control pollution, is important to treated wastewater and the water that is generated from the livestock units before discharge them in the sea water, because they are a risk factor of pollution.

**Key-words:** water, quality, pollution, analysis, samples, treatment

**Introduction**

The control of water quality characteristics is an essential element for the solution of various problems related to water resources management, because the water is a natural resource, so its quality must be protected, managed and treated.

The urban growth and industrialization of these areas is accompanied with increasing the pollution in aquatic environments. The development of human society has constantly an extensive use of water in its various fields like industry, agriculture, trade and transport, electricity, sanitation and potable water primarily.

Studies conducted in recent years show that pollution of bays, deltas and lagoons are in a concerning level. (Valdas D & Real E: *India Journal of Marine Science* 2004, 33(4): 338-345)

Because of limited hydrodynamic as the relatively limited environments communication with the seas, those accumulate for a long time with pollutants with different origin and cause problems in: biota, food chain, tourist activities, in other economic activities (as navigation).

(Apitez S, Barbanti A, Bernstein A, Bocci M, Delaney E and Montobbio L: 2007 Vol. 24: 128-141)

In the multi-functional bays (as is Durres bay) pollution problem becomes even more concerning because, for various purposes, are required physical, chemical and biological parameters of the water.

In the Durres Bay have been identified 3 main sources of pollution:

1. the port activity, although complete with an aquarium, has relations with the whole beach, due to the mixing of the water column, contributing mainly to contamination with heavy metals and hydrocarbon
2. The beach through sewage
3. Agricultural activities carried out especially in the field of Kavaja.

The Durres bay is very dynamic due to the activities that take place in it. It contains the largest port and the largest beach in the country.

In summer the population reaches about 300 000 inhabitants.

In the beach area there are more than 1300 buildings, which serve as a summer house, with a very complicated urban problem.

Given the concentration of buildings and contributions of all categories in the ecological balance of the Bay, we see that the parameters are almost in every case higher than are definite in the legislative package. Chemical-physical qualities and especially microbiological the Durres bay waters are so aggravated as any investment in the control of contaminants would have a high "efficiency" and will improve a sufficient water quality.

The purpose of the study is the assessment of water pollution in the Bay of Durres from urban wastewater discharges and livestock, with the aim of identifying risk factors and improving water quality.

The treatment of used water (wastewater) is a process of improvement and / or purification, eliminating some or all pollutants and discharging it almost clean, in the surroundings. In many countries of the planet health problems and diseases caused by wastewater discharges as are either not addressed in the current accepted rate. (*Livingston JR: 2001: 319.*)

To realize this purpose and to assess the pollution of the water in the Bay of Durres from wastewater discharges, including the one generated from livestock units. Monitoring expeditions were carried out in the coast line of Durres Bay. It was appointed 2 sampling points: the first point named Currila in the north suburb of the city of Durres and the second point named Plepa in the estuary of the wastewater and livestock water discharges, in the south of Durres city and was analysed physic-chemical indicators in May and August 2011.

### **Material and methods**

In the context of environmental protection, control of natural water quality and wastewater discharges, takes primary importance. There are a number of methods for sampling, sample processing and measurement of their quality characteristics. In order to physic-chemical analysis data for the quality of the water was carried out in Albania, to be comparable with international ones, a special importance is the approximation of the sampling methods and physical and chemical analysis, and international ones. (*Kennish MJ: 997: 524.*)

When should be characterized a water volume, a bottom sediment or sludge, generally it is impossible to analyze all measures is therefore necessary to take samples. Samples collected should be as representative of the whole and must take all necessary measures to ensure, to the extent possible, that the samples do not undergo any change in the time interval between sampling and analysis. Sampling multistage systems, such as water containing solid matter suspended or organic liquids that do not mix, can pose special problems.

There were 2 sampling points predefined:



- 1<sup>st</sup> point named Currila in the north suburb of the city of Durres 2 points in the estuary of sewage discharge; Plepa and Golem
  - 2<sup>nd</sup> point named Plepa in the estuary of the wastewater and livestock water discharges, in the south of Durres city
- Sampling was carried out in in May and August 2011, taking into account the seasonal effects,
- Were analyzed bacteriologic, physical and chemical indicators such as: Streptococcus Faecalis, Escherichia coli temperature, pH, conductivity, salinity, dissolved oxygen (DO), N-NH<sub>4</sub>, N-NO<sub>2</sub>, N-NO<sub>3</sub>, P-PO<sub>4</sub>, and COD, BOD<sub>5</sub> (only for samples in the estuary of sewage discharge), according to standards and methods based primarily on modern methods of book "Selection and development of methods of sampling and analysis of environmental contaminant elements the sampling of waters" prepared by the Institute of Environment and the manual "Sampling and analysis methodologies for the Monitoring of eutrophication strategy of MED POL", in the Laboratory of the Department of Agro-Environment and Ecology, Faculty of Agriculture and Agro-Environment, University of Agriculture in Tirana.

### Results and discussion

The results of bacteriologic, physic-chemical analysis in the estuaries of the waste water discharges showed evident differences regarding the EU normative, specifically the bacteriologic analysis.

The level of *Streptococcus Faecalis* resulted  $3.1 \times 10^3$  and  $9.3 \times 10^3$  MPN/100 ml water (May/August) in the Currila point, versus  $3.5 \times 10^4$  and  $9.8 \times 10^6$  MPN/100 ml water (May/August) in the estuary of the wastewater discharges (Plepa point), while the reference parameters of EU for the bathing water quality is  $2 \times 10^3$  MPN/100 ml water.

The level of *Escherichia coli* resulted  $1.5 \times 10^3$  and  $3.4 \times 10^3$  MPN/100 ml water (May/August) in the Currila point, versus  $2.3 \times 10^5$  and  $9.3 \times 10^6$  MPN/100 ml water (May/August) in the estuary of the wastewater discharges (Plepa point), while the reference parameters of EU for the bathing water quality is  $1 \times 10^4$  MPN/100 ml water.

Table 1. Currila point Results

Currila point	<i>Streptococcus Faecalis</i> MPN/100 ml		Classification	<i>Escherichia coli</i> MPN/100 ml		Classification
	EU normative	Result		EU normative	Result	
May 2011	$2 \times 10^3$	$3.5 \times 10^3$	Outside norm	100	$1.5 \times 10^3$	Outside norm
August 2011	2000	$9.3 \times 10^3$	Outside norm	100	$3.4 \times 10^3$	Outside norm

Table 2. Plepa Point Results

Plepa point	<i>Streptococcus Faecalis</i> MPN/100 ml		Classification	<i>Escherichia coli</i> MPN/100 ml		Classification
	EU normative	Result		EU normative	Result	
May 2011	$2 \times 10^3$	$3.1 \times 10^4$	Outside norm	100	$2.3 \times 10^5$	Outside norm
August 2011	2000	$9.8 \times 10^6$	Outside norm	100	$9.3 \times 10^6$	Outside norm

As seen in the following graphs (Table Currila point Results and Table Plepa Point Results) the seasonal influence is evident. Also we concluded that the showed difference from Currila waste water discharge in the Plepa discharge is because the south of Durres city (Plepa point) is closer to rural areas, so the wastewater generated from livestock units has a big influence the pollution of the sea water in the Durres Bay.

According to the negative effects of excessive amounts of the bacterial elements cast in Superficial coastal waters are considerable; they cause increased total biomass of algae, often, toxic algae, reduce herbaceous layers last sea coral habitats, significantly reduce marine biodiversity and worsen the fishing trade.

Biodegradable organic substance and nutrients (fecal, food waste, manure, food industry waste) are subject of the decomposition processes, releasing chemical elements, generally feeder for phytoplankton and the process of aerobic decomposition consume O<sub>2</sub> in water (*Thornton JA, McComb J, and Ryding SO: 1995: 205-224.*)

The Bacterial, Chemical-Physical qualities of Durres bay waters are aggravated and any investment in the control of contaminants would be a high "efficiency" and will improve sufficiently the water quality.

Discharge of wastewater, including the one generated from livestock units, without any preliminary treatment in surface waters in the Bay of Durres and in all the beaches of Albania has high impact pollution.

We conclude that, to control the pollution is a necessary the treatment of wastewater before discharging in the sea, as a risk factor in the level of contamination the Durres bay.

Even studies in recent years that have been carried out on the beaches of Durres Bay pollution testify to their very high. Results show that 85% of beaches are classified in D Category - very polluted, where urgent action to improve the situation, 14% in C Category - sufficient quality, and only 4% in B Category - good quality.

Even under the EU Bathing Directive, in some areas should be established tables that prohibit bathing, because the results are alarming.

This assessment requires urgent measures to improve the situation because the risk of public health and biodiversity in Durres Bay is very high.

### Conclusions

- For the Water protection, the effects of urban wastewater discharges and livestock must be controlled in continued among others.
- To check this contamination, definitely, it's should be carried out the wastewater treatment before discharge into the sea.
- Sewage spill into the sea untreated constitutes a risk factor in Durres Bay pollution level.

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## ENERGY FROM DAIRY FARMS

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### Abstract

In the future farmer's will have a problems whit amount and manipulation of manure from farms. Aim of paper is to present the potential of producing electric and thermal energy from cattle manure in the Republika of Srpska (RS) and to solve problems.

Dairy farmers have problems how to reduce the cost of milk production and this is way how to help them to provide a new sources of income. On the other hand, increasing the number of animals on farms, leading to problems with increasing amounts of manure and its proper storage. In front of us is adoption of relevant EU directives, which regulate this field, it will mean additional costs to agricultural producers who comply with these conditions.

The possibilities of energy production, at the cattle farm in Republika of Srpska, it was found that can produce about 0.6 GWh of electrical energy and 0.97 GWh of thermal energy per day if we used all manure.

This can represent significant revenue for the farm, but also a significant contribution to the preservation of the environment. Republika of Srpska need to change legislation in these subjects and provide a same price like in other country.

A problem is because a legislative treats looked on this projects like energy plants, not the agricultural aspect, which significantly increases the price of building these plants.

It can be concluded that we are finally reconciled ecology and food production who are often controversial.

**Key words:** *biogas, ecology, energy, manure, dairy farms.*

### Introduction

The country, member of the European Union (EU) have brought the relevant regulations governing this area "Nitrates Directive (Council Directive 91/676/EEC)." This is a problem with increasing of production. The other side there are also recommendations of the FAO in its report "Livestock Long Shadow," announced the emission of harmful gases into the atmosphere from livestock production involved about 18% more than the transport industry (Mitloechner, 2012). Renewable energy sources are an integral part of the EU fights against climate change while contributing to economic growth, job creation and increasing energy security. Biogas production from agricultural biomass is of growing importance as it offers considerable environmental benefits and is an additional source of income for farmers (Balsari et al., 1983).

To overcome all these many member states of the European Union have put in front of themselves clear objectives, which can be achieved by applying the procedures for the rational utilization of organic matter, and that applies to manure. One of these procedures is certainly the production and use of bio-gas from organic agricultural waste (manure), anaerobic digestion process. Manure from all types of farms, which can still add and crop waste, straw, silage corn, is a biodegradable and the process takes place without any special

additives. With this method may be enough to contribute to the sustainability of the farm business. This primarily refers to reducing the cost of electricity and thermal energy, which can be used on the farm or sold to the network, which would provide an economic benefit. Processed material could be used in agriculture and ecology represents even better material because it reduces the possibility of contamination of underground water (Amon et al., 2006). Modern technologies require more electricity, and thus increases the price of inputs in the production of milk. The other side the price of milk remains the same or most often decreases. In order to overcome these problems and ensure the normal operation of producers of milk usually increases the number of cattle per farm. The other side comes up to increase the amount of manure production according to unit area and according to producer facilities.

As a particular aspect of this production and environmental component, because with this kind of manipulation leads to decrease the effect of "greenhouse". Traditional disposal of manure fertilizer on the open release huge amounts of gases that influence this effect (Budimir & Prskalo 2012).

Anaerobic decomposition processes of organic matter were known even in the 18<sup>th</sup> century, but the first application of anaerobic fermentation, as a method for processing of faeces, began only in early 20<sup>th</sup> century (Deublein et al., 2008). Since then, the application of anaerobic digestion is spreading rapidly, developed in the biological and chemical terms. Environmental pollution and the need for renewable energy sources have increased interest and so that more funds invested in scientific - research work, so that in many countries build more plants using this method of organic decomposition. In this particularly advanced: Germany, Denmark, Holland, Switzerland, and Belgium, that country with developed agriculture production or livestock.

Digestion of this type of waste could significantly reduce parasites and pathogenic bacteria by 90% and thus protect underground sources of water, and reduce the disposal of industrial waste that causes pollution of water and soil (Sahlstrom, 2003).

Biogas obtained by anaerobic digestion from biomass contains: 50 - 60% methane (CH<sub>4</sub>), 35 - 40% carbon dioxide (CO<sub>2</sub>) and up to 5% mixture of hydrogen, nitrogen, ammonia, hydrogen, CO, oxygen and water vapor (Budimir & Prskalo, 2012).

Pure methane has a calorific value of 9100 kcal / m<sup>3</sup>, and the basic characteristics of biogas with 55% methane are: calorific value of 4800-6900 kcal / m<sup>3</sup>, specific gravity 0.86 (air = 1), the energy potential of 5.5 to 7.5 kWh / m<sup>3</sup>.

The total potential of the Republic of Croatia in 2003. was estimated at 1.2 million m<sup>3</sup> of biogas (Kralik, 2007.). Since most of this quantity of waste to manure from cattle farms, and is 59%, while the rest goes to waste from pork and poultry farms, around 15%. Other wastes from other animals. Adding production from plant waste or other organic waste utilization plants increases (Kricka et al., 2009).

The potential production of biogas in pig and cattle farms in the Vojvodina is about 9.5 millions m<sup>3</sup>. The total possible amount of electricity generated, from pig and cattle farms is around 20 GWh per year, and heat for about 8 GWh (Tescic, 2008).

In Bosnia and Herzegovina, the advantage would be if all the manure from dairy farms, a day can produce about 1.5 GWh of electric and about 2 GWh of thermal energy (Budimir & Prskalo, 2012). The same authors research that the government must change a policy in renewal source of energy, in udders case they can't be a profitably in this moments.

### **Material and Methods**

For the paper preparation were used data of the Statistical Yearbook (2011), the Statistical Office of the RS.

On this basis, a potential production of manure is calculated. Of the total amount of manure based produced on the farm, most are used to produce biogas.

Based on the literature and authors' own research, calculated the possibilities of electricity and thermal energy in the Republic of Srpska.

On the farm ZZ "Liva " have a first bio-gas plant and used a result of production electric energy. The plant was put into the work end of 2011. Installed power is 45 kW/h of electricity and we used to compare a theoretical a practice aspect of producing energy.

### Results and discussion

Further calculation of available amount of manure to produce biogas, will be based on the number of cattle - UG (conditionally throat is converted units of 500 kg of animal weight).

The quantities of straw is one of the factors that influence the dry matter content of manure, and from whose percentage depends on the amount of the produced biogas. In bovine manure dry matter percentage is quite low and theoretically ranges 5-12%, but this value depends also of cattle, of feeding and manure removal, housing conditions and the like.

A laboratory examination of samples taken from the fermenter was found that organic matter content was 8.97% (the calculation we use the value 9%).

According to Statistical Yearbook (2011), the numner of cattle in RS amounts 235.000. From this number a 129.000 is cow and pregnant heifers. We can assume that the difference is the number of heads and cattle fattening bulls and calves. To make us easier for calculation we will tolerate that the number of heads from yearbook equal to the number of standard livestock heads.

The average daily amount of manure they produce 1UG is 50 kg. It follows that the total daily amount of manure that produces in RS is 11.750 tons.

Production of biogas from manure per conditional throat adult animals ranges from 0.9 -1.6 m<sup>3</sup>/day (Budimir & Prskalo 2012). What affects the amount of the gait, bound or free. Has greatly influenced the litter, which can increase the production of biogas and 50% if it is used in the facility. This was particularly true in the straw of cereals. When using straw as bedding, the recommendation is to use chopped straw to a length of 2 - 5 cm, as this improves the process of decomposition of organic matter and increases the production of biogas.

If we take an average production of bio-gas is 1.25 m<sup>3</sup> from UG from manure, we can calculated that in RS daily productions of biogas is 293.750 m<sup>3</sup>. this amount of gas going to atmosphere and cause a pollutions air.

Energy potential of 1m<sup>3</sup> of biogas is:  $E_{pt} = 6 \text{ kWh} / \text{m}^3$

Should be noted that the above potential of energy relations to the total energy that can be obtained from the biogas with provided that, as already mentioned, when cogeneration gains: 35% electricity, 55% heat and 10% are losses in cogeneration module.

Total daily energy potential (electric and thermal energy), therefore is:

$$E_{\text{total}} = 293750 \text{ m}^3 \cdot 6 \text{ kWh} / \text{m}^3 = 1762500 \text{ kWh} \text{ or } 1.76 \text{ GWh energy.}$$

$$E_{\text{total}} = E_{\text{el}} + E_{\text{heat}} + E_{\text{losses}}$$

From the ratios presented above are gains that day products:

$$E_{\text{el}} = 616875 \text{ kWh} - \text{electric energy or } 0.6 \text{ GWh}$$

$$E_{\text{heat}} = 969375 \text{ kWh} - \text{heat energy or } 0.97 \text{ GWh}$$

### Conclusion

Republika of Srpska has the some regulations in renewal source of energy. They aren't a best but they exist and they can use for a new policy in this subjects, special for more price energy and new subventions for agriculture producers who want to invest in this projects. On the other hand is possibly for this projects use money from EU funds.

If we calculated on research we can see that possibility exist in new source of energy. Based on all the above we see that the production of electricity from manure can increase farm profitability and realize additional income. There is also the thermal energy that can be further utilized for other ongoing activities (greenhouse production, drying, heating facilities, etc.). With the increasing use of thermal energy we will increase profitability of the plant.

On the other hand this reduces emissions of harmful greenhouse gases into the atmosphere and takes part in environmental protection. The possibly productions in RS only from cattle manure is about 0.6 GWh of electrical energy and 0.97 GWh of thermal energy per day. If we calculate possibly productions per year we'll get a big amount of energy what we losing in this moments. Of course we don't need too use all manure but definitely it should solve a problems whit his manipulations.

As a conclusion we can say that we are finally reconciled ecology and food production who are often controversial.

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**CLIMATE CHANGE AWARENESS AND SMALL SCALE MAIZE FARMERS IN  
MPUMALANGA, PROVINCE, SOUTH AFRICA**

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**Abstract**

Climate change is possibly the greatest environmental challenge facing the world this century. The impact of climate change is a reality and it cuts across all climate-sensitive sectors including the Agriculture sector. It was well documented by several scientists, Intergovernmental Panel on Climate Change and other experts that climate change threatens sustainable economic development and the totality of human existence. This study was conducted in Nkangala District, Mpumalanga province. Mpumalanga province remains the largest production region for forestry and the majority of the people living in Mpumalanga are farmers and they have contributed immensely to promote food security. However, due to the impacts and threaten by climate variability and change which resulted into shortage of food production and changes in the rainfall pattern. It was noted that there is a need for climate change awareness across the agriculture sector including farmers. Random sampling technique was used to select two hundred and fifty one farmers to be interviewed. The questionnaire was administrated to farmers and included matters relating to climate change awareness and agronomic practices including maize production. Data was captured and analysed using software package for social science (SPSS version 20). Descriptive analysis was used to describe data and Univariate regression analysis was conducted to demonstrate the relationship and association of variables. It was noted that the majority of farmers in this province need capacity building and also climate change awareness initiatives which would assist these farmers to build the adaptive capacity, increase resilience and reduce vulnerability.

**Keywords:** *Climate Change awareness, Nkangala District Mpumalanga Province South Africa, Small Scale Farmers and Maize Production.*

**Introduction**

The impact of climate change varies globally; however, the problem and the challenges of climate change are becoming more threatening to sustainable economic development and the totality of human existence (Adejuwon, 2004). Small-scale farmers suffer the most because of their dependence on rain-fed agriculture, limited financial capacity, low adaptive capacity, high dependence on natural resources, inability to detect the occurrence of extreme hydrological and meteorological events due to low technology adoption, limited infrastructure, illiteracy, lack of skills, level of awareness and lack of capacity to diversify (Kurukulasuriya & Mendelsohn, 2006a). Maize constitutes about 70 percent of grain production and covers about 60 percent of the cropping area in South Africa. In addition, maize is the main staple food in Southern Africa, and maize production in the country constitutes about 50 percent of the output within the Southern African Development Community (SADC) region (Durand, 2006). A considerable number of studies have been done to investigate the impact of climate change on yields of grain crops such as maize under controlled experiments (Du Toit *et al.*, 2002; Kiker *et al.*, 2002; Durand 2006).



There is very little awareness on climate change in the developing countries (IPCC, 1996). The overall objective of the paper is (i) to create awareness through which farmers can understand the impact and the threats that climate change pose within the agriculture sector. (ii) Build adaptive capacity and reduce vulnerability facing small scale farmers in Mpumalanga Province. This will enable small-scale maize farmers in Mpumalanga province to have basic understanding about the impact of climate change in their areas. This will increase maize production as well as their income for better living.

### Materials and Methods

This paper used quantitative design as a well detailed structured questionnaire written in English language as part of the data collection methods. The questionnaires consist of a logical flow of closed ended questions, which address issues related to climate change, agricultural production, yields etc. Data was collected through face-to-face interviews with the farmers and also the help of the extension officer where 251 questionnaires were administered in the study area. The study was conducted in Emakhazeni local municipality within the Nkangala district municipality in the Mpumalanga province of South Africa. Stratified sampling technique was used to select two hundred and fifty one farmers to be interviewed. Data was captured and analysed using software package for social science (SPSS version 20). Descriptive analysis was used to describe data and Univariate regression analysis was conducted to demonstrate the relationship and association of variables. The following econometric model was used to determine association of variables:

$$W_i = \beta_0 + \beta_1 X_i + \epsilon_i$$

$W_i$  is the dependent variable value for person  $i$

$X_i$  is the independent variable value for person  $i$

$\beta_0$  and  $\beta_1$  are parameter values

$\epsilon_i$  is the random error term

The parameter  $\beta_0$  is called the intercept or the value of  $W$  when  $X = 0$

The parameter  $\beta_1$  is called the slope or the change in  $W$  when  $X$  increases by one

### Results and Discussion

**Table 1.** Summary characteristics of household sample in the towns.

Towns	Number of Households	Percentages
<i>Number of Households per Towns in Emakhazeni Local Municipality</i>		
Belfast	50	19.9
Dullstroom	45	17.9
Machadodorp	40	15.9
Noodgedarht	30	12
Stoffberg	30	12
Watervalboven	30	12
Wonderfontein	26	10.3
<b>Total</b>	<b>251</b>	<b>100</b>

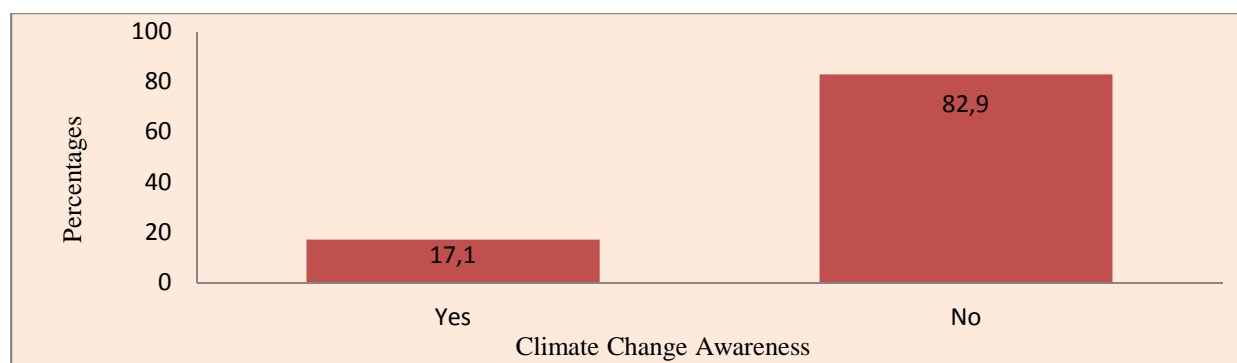


Figure 1. Households Climate Change Awareness

As shown in (figure 1) 82.9 percent of the households are not aware about climate change and only 17.1 percent households indicated that they are aware of climate change. This is not surprising because the majority of households indicated that they are not aware about climate change issues due to: (a) lack of information especially climate advisory, (b) lack of education and lack of assistance from the extension officers. According to Olayinka *et al.*, (2013) awareness of the various causes of climate change is generally below average and less than 50 percent however sees it in terms of reduced agricultural productivity or ozone layer depletion.

Table 2. Univariate regression analysis of potential determinants of climate change awareness and maize production

Variable	Total	(%)	OR [95%CI]
Females	52	20.7	1.00[0.508 – 2.711] 1
Age	251	100	0.99 [0.440 – 2.567] 1
Occupation	251	100	1.10 [0.675 – 3000] 1
Education	251	100	1.01[0.599 – 2899] 1
Source of income (Yes)	179	71.3	0.97[0.127 – 2.112] 1
Climate change info	251	100	1.53[0.76 – 3.555] 1
Extension Service	251	100	1.50[0.68 – 3.44] 1
Importance of info	251	100	1.12[0.576 – 2.666] 1
Quantity of harvest	251	100	101[0.11- 2011] 1

OR= Odds ratio; 95%CI = 95% confidence intervals; 1< = no association; 1> = association

As shown in Table 2, there is association among the following variables: gender, age, occupation, education, source of income, information on climate change, extension service, importance of information on climate and quantity of harvest. This is supported by the fact that their estimate values are more than 1 at 95% confidence interval.

Gender (Female) had significant impact on the level of climate change awareness. According to Table 2, the odds of climate change awareness are 1.00 percent higher for female households than male households. It is widely recognised that climate change does not affect

people equally (UNEP, 2011). A study done by Nhemachena and Hassan (2007) acknowledged women contribution in agricultural sector in relation to climate change especially women's leadership in natural resource management in developing countries.

Age is another significant variable which is associated to climate change awareness. According to Table 2, the odds of climate change awareness are 1.00 percent higher across all age categories. This is not surprising because climate change awareness is made across all age categories even in the schools and out of the school through the use of fliers, posters and many medium. This conforms to the finding of Olajide *et al.*, (2011) who found a significant association between age and knowledge of global warning among undergraduate students of Obafemi Awolowo University (OAU), Ile Ife, Nigeria. According to Bayard *et al.*, (2007) age is positively related to some climate change adaptation measures that are related to agricultural activities.

According to Table 2, the odds of climate change awareness are 1.10 percent higher across all occupations. The study shows that occupations of the respondents have significant impact on the level of climate change awareness. This could be from the fact that some farmers take farming as a full time and some farmers take farming as part time activity but in each case, they all come across awareness either through indigenous knowledge or at their various place of work through adverts. According to Adebayo *et al.*, (2003) occupation has a significant association with awareness of climate change.

According to Anley *et al.*, (2007) improving education and employment is key to stimulate local participation in various adaptation measures and natural resource management initiatives. It was further emphasised by Maddison (2007) that educated and experienced farmers are expected to have more knowledge and information about climate change and adaptation measures to use in response to climate challenges.

According to Table 2, the odds of climate change awareness are 1.00 percent higher across for households who rely on maize as source of income than households who don't rely on maize as source of income. From the study, farmers who rely on maize as a source of income have no other job or extra source of income, than farming activities, thus, they are involve and concern about their environment in relation to their faming activities because they need to provide for the household thereby tends to be more aware of the climate change as an environmental factor responsible for production, unlike farmers who have other source of income apart from farming.

According to Table 2, the odds of climate change awareness are 1.53 percent for households with climate change information. This is shows that climate change information is significantly associated with awareness level of climate change. This is not surprising because a study reported by Luseno *et al.*, (2003) said the more the farmers had access to extension services and information about climate change, the more they adapt to climate change. From this study, the amount on climate change information at farmers' disposal determines the level of awareness of climate change.

According to Table 2, the odds of climate change awareness are 1.50 percent for households with access to extension services. The study shows that access to extension services significantly affects awareness to climate change. Extension services provide an important source of information on climate change as well as agricultural production and management practices. Farmers who have significant extension contacts have better chances to be aware of changing climatic conditions and also of the various management practices that they can use to adapt to changes in climatic conditions. This is also in conformity with Mandleni (2011)

that formal extension positively and significantly affects awareness to climate change and adaptation.

According to Table 2, the odds of climate change awareness are 1.12 percent for households who recognise the importance of climate change information. According to Nhemachena and Hassan (2008), farmers that perceive change in climatic conditions and farmers who have access to climate change information have higher chances of taking adaptive measures in response to observable changes. There is no doubt that climate change awareness plays a very important role in the agriculture sector and if farmers have information in their disposal, the majority of these farmers can make good decisions as it was noted by Mpandeli (2005).

### **Summary and Conclusion**

Due to prevailing problems associated with changes in weather patterns such as high temperatures, changes in rainfall patterns and effect of greenhouse gases (GHGs), which has resulted in low crop production, food insecurity, low income for farmers, there is a need to investigate whether small scale maize farmers are aware of climate change. So this study will enable small scale maize farmers and households in Mpumalanga province to understand the meaning of climate change, by creating awareness through which households and farmers can cope with climate change. This will improve climate change adaptation and thus increasing maize production as well as income for households and farmers.

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**REGIONAL GEOMORPHOLOGICAL MAPPING OF MONTENEGRO:  
LANDFORM GENESIS AND PRESENT PROCESSES**

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**Abstract**

As a contribution to the joint geomorphological research carried out by Ghent University and the University of Montenegro (<http://geoweb.ugent.be/physical-geography/research/western-balkans>) the main geomorphological features of Montenegro will be characterised and mapped. The main geomorphological regions were identified based on past research in the different geomorphological regions. A polje (Njeguse), a canyon (Kanjon Starobarske) and a debris fan and fluvial terraces (Moraca - Podgorica) will be mapped in detail using GPS measurements, topographic maps (1:25 000) and GIS software. Additionally, a large-scale geomorphological map of the Montenegrin territory will be created using existing literature, DEM, GIS software and soil samples. Homogeneous geomorphic units will be mapped using the geomorphon approach. Another aspect of this research concerns the formation of Skadar Lake, since existing literature shows contradictory theories: subsidence, or a rias coast that was isolated by the Bojana alluvial deposits, or a combination of both. Therefore, this region (including Bojana river alluvial plain and Ulcinj coastal dunes) will be subjected to sedimentological research. The resulting maps and sedimentological data will be used to fully understand the different formation processes of the present-day geomorphology. Furthermore, measurements and photographs will be used to assess the influence of land use changes on gully erosion.

**Introduction**

The fieldwork is performed in 2013 in order to map the most important geomorphological landforms in Montenegro. With these maps, an interpretation will be made of the landform genesis in addition to a clear visualization of the different geomorphological regions and features in the country. Furthermore, research is dealing with the main current erosion processes.

From a geographical point of view, three regions more or less homogenous – concerning climatology, lithology, hydrography and vegetation – can be described. Starting from the south, the Mediterranean coastal part (Coastal Montenegro), the Submediterranean central part (Central Montenegro) and the mountainous northern – north eastern part (Northern Montenegro) are discerned.

**Geomorphological regions**

*Coastal Montenegro.* Three north west – south east oriented (typical for Dinaric alps) units are aligned next to each other: the High Karst consists of Mesozoic carbonates, the Budva zone consists of Triassic limestone and the Dalmatian zone represents different linear structures consisting of Cretan-Eocene limestones (anticlines) and flysch deposits (synclines). The combination of these three zones results in quite a differentiated topography.

*The High Karst zone and Skadar Lake.* This limestone structure has been unequally uplifted; the altitude varies between 1300 m above Kotor to 200 m in the east. Apart from there, the

topography is karstic, with many dolines, sharp ridges and residual reliefs in the weathered limestones. On the bottom of the dolines and karstic depressions, small villages, farms and rural communities are concentrated. The High Karst zone consists of limestones and dolomites, heavily fractured by tectonic events.

In the southeastern part of this region the High Karst Zone borders Skadar Lake. Skadar Lake lays in a graben, filled with alluvial sediments from the Drin River, the longest river of Albania. For this reason, the lake is generally quite shallow (4 m), except for the part where the lake meets the valley of Rijeka Crnojevica. In this area the bottom of the lake gets very ragged with dolines, uvalas and sub-lacustrine sources (Nicod, 2003). Another theory about Skadar Lake states that the area was originally part of the Adriatic Sea (similar to Kotor Bay). The only connection with the Adriatic Sea would have been closed by a combination of alluvial sedimentation of the Drin and supply of aeolian from the coast to form dunes (Nicod, 2003).

*The inland depression - Niksic Polje.* In the inland depression 3 different regions are discerned: the plain of Podgorica on the debris fan of the Moraca River, the Zeta-valley and Niksic polje.

*Durmitor.* The Durmitor massif is one of the highest in the Dinaric Alps and very characteristic for its glacial and karst morphology and deep canyons. It dominates the surrounding karst plateaus: Jezera in the east and Piva in the North West, which are demarcated by the canyons. Durmitor consists of Triassic and Jurassic carbonates and late-Cretaceous flysch. Tectonic uplift is estimated to be quite slow (6 m/ka), with clear traces of past activity. The Bobotov Kuk fault is clearly visible in the relief, determining the orientation of the cirques and canyons. In the massif, numerous cirques are to be found, formed in the flysch rocks on the one hand and glacial karst cirques formed in the carbonates and dolomites on the other hand (Nicod, 2003).

*Prokletije.* Many authors have studied the physical-geographical characteristics of this area. Cvijic (1921) called attention to the geographical individuality of the Region, with special emphasis on the Prokletije mountain group. The major part of this massif lies within the territory of Albania and a smaller part in Kosovo, but still a considerable part of the area lies within Montenegrin territory. This part is one of the National Parks of Montenegro, alongside Biogradska Gora, Durmitor, Lake Shkodra and Lovcen. The Prokletije mountains are the highest massive of the Dinaric Alps, reaching a height of 2694m (Maja e Jezercë) in Albania and containing Zla Kolata, the highest peak of Montenegro at a height of 2534 m. The area has only recently been explored due to political instability and poor accessibility.

Across the border, in Albania, some still active glaciers were discovered on 15 september 2007, making it one of the southernmost glaciers of the European continent. In the Montenegrin part plenty of cirques, glacial valleys (e.g. Ropojana and Grbaja) and other periglacial evidences are found (Milivojevic *et al.*, 2008).

*The northern crystalline hills.* The only region not yet covered in this overview is the northern of Montenegro, including Biogradska Gora and the municipality of Bijelo Polje. Paleozoic clay and sand, Triassic red sandstone and dark ophiolites, are found (Ager, 1980). This geology is reflected in the landscape, where smooth hills and valleys are formed due to higher vulnerability for lateral erosion. Furthermore, this part belongs to the catchment area of the Black Sea and was consequently less influenced by sea level changes in the Mediterranean.

### Geomorphological phenomena

*Karst.* As Radulovic (2013) writes: “Karst is a geological term which refers to a set of specific morphological forms of landscape that are the result of interaction between a number of factors, primarily water and water-soluble rocks. Therefore, karst forms are developed only in terrains made of soluble rocks, commonly limestones and dolomites, but also in terrain made of gypsum, anhydrite and halite rocks. Due to the solubility of carbonate rocks (limestones and dolomites), tectonic faults are expanded and secondary porosity of rocks is increased.” A major part of Montenegro is part of the Dinaric karst. This part geologically consists of limestone and dolomite sedimentary rocks, formed in favourable climatic conditions. Due to tectonic activity, folding, faulting and overthrusting increased the porosity of the rock, intensifying the karst processes. That caused the present-day very complex karst landscape, marked by karren, sinkholes (dolines), uvalas, poljes, dry valleys and caves (Radulovic and Radulovic, 1997).

*Canyons.* Very impressive in the karst landscape, numerous steep and narrow canyons deeply incised the rocks. It is safe to state that such deep incisions (500m to over 1000m) are unlikely to be caused only in Quaternary times. Most probably, these incisions are mainly caused by a more extreme sea level lowering event such as the Messinian Salinity Crisis. Canyons are often – if not always – part of a karst landscape because of the strong resistance of carbonate rocks to erosion, resulting in a vertical incision and thus narrow valleys (Djurovic and Petrovic, 2007).

*Rias.* The term ‘ria’ is used to describe a former river valley system developed in a high relief coast that is drowned by sea level rise. The resemblance of the morphology above current sea level to a fjord could cause some confusion, but as a ria coastal system has nothing to do with glacial erosion, the morphology of the drowned parts is different (Castaing and Guilcher, 1995).

In Montenegro, Kotor Bay (*Boka Kotorska*) provides a typical example of this phenomenon. These valley systems were formed before the Holocene due to glacial and interglacial sea level changes and especially during the Messinian Salinity Crisis (ca. 5,5 million years ago), when the Mediterranean Sea was nearly completely dessicated because of tectonic and glacio-eustatic uplift of Gibraltar Street. As a result, sea level – thus erosion basis - lowered with probably more than 1000 meters (Krijgsman *et al.*, 1999) which allowed regressive erosion in the landscape. The result is a very deep incised morphology under the current sea level by a river flowing in NE-SW direction, orthogonal to the orientation of the anticlinal structures with its tributaries, parallel to the anticlines. In the hard carbonates, narrow and steep valleys were incised while in the soft flysch layers the river and tributaries formed wide valleys, causing the NW-SE orientation of the bays (Magas, 2002).

*Debris fan and river terraces.* Many rivers developed in the Dinaric karst, often incising deep, narrow canyons, form wide alluvial fans when reaching alluvial plains close the sea (Djurovic, 2007). Upstream of Podgorica, the Moraca River has left a large debris fan.

During glacial periods in the Quaternary - meaning a lower sea level - the alluvial plain and fan were incised by the river due to regressive erosion. When sea level rose, aggradation took place, leaving a new layer of river sediments. In the alluvial plain of Podgorica and the debris fan, three or four (Keukelaar *et al.*, 2006) fluvio-glacial terraces can be recognized in the landscape caused by the interaction between the aggradation of the Moraca and Cijevna rivers and tectonic subsidence of the region (Nicod, 2003).

*Coastal Dunes.* Ager (1980) stated that an inlet of the Adriatic Sea would have been closed by dune formation as an explanation of Skadar Lake. Supporting for this theory is the present-day location of dunes in the area of Ulcinj (near the contact of the alluvial plain of Bojana river with the Adriatic) and the deep incised morphology in the north-western part of Skadar



Lake, very similar to the ria of Boka Kotorska. In addition to these findings, Google Earth shows the enormous alluvial cone from the Bojana River. More recent references are few but point out the fact that the lake is formed in a tectonic depression. The lack of more recent references about this subject makes it a potentially very interesting research object.

*Glacial geomorphology.* During several cold periods in the past a considerable part of Montenegro was covered by ice caps. The maximum extent of these ice caps is believed to be reached during the MIS (Marine Isotope Stage) 12, in the Middle-Pleistocene (ca. 470-420 ka). At that time, the Durmitor, Sinjajevina, Moraca, Magnanik and Prekornica massifs were covered by one huge ice cap with an area of nearly 1500 km<sup>2</sup>. More recently, valley and cirque glaciers were formed in the Younger Dryas and some up-valley glaciers during the early Holocene (Hughes *et al.*, 2011). In Prokletije Mountains, three glaciation events with valley and cirque glaciers are recognised but no numerical dating has been done yet. Nevertheless it is assumed that the maximum glacial extent in this area took place in the Early- or Middle-Pleistocene, while the second event probably happened during the Last Glacial Maximum and the last during the Younger Dryas. The Orjen massif, close to the Adriatic coast (north of Herceg Novi) was regularly covered by an ice cap too (Milivojevic, 2008).

Since mapping and detailed description of the Durmitor and Prokletije regions with special attention for glacial geomorphology has already been done, these phenomena will not be part of the detailed geomorphological mapping.

### **Current erosion**

Many factors have influenced the erosion processes in Montenegro. The most significant factors are the area's climate, relief, geological substrate and pedological composition, as well as the condition of the vegetation cover and the land use (Spalevi , 2011). Water erosion is the most important erosion type. It is caused due to precipitation and consecutive runoff primarily, but also by fluvial erosion in water streams (Kostadinov *et al.*, 2006). Kostadinov *et al* (2006) summarized erosion in Serbia and Montenegro using the categorization of Gavrilovi (1972). According to Spalevi (2011), Kostadinov (2006), Lazarevi (1996), water erosion has affected 13,135 km<sup>2</sup> or 95% of the total territory of Montenegro (13,812 km<sup>2</sup>). Given the extreme precipitation values in some parts of the country (the highest of Europe) the influence of this erosion type on the landscape is enormous. The erosion forms are often characteristic for karst regions, although other forms are observed as well. Following the categorization, almost half of the territory of Montenegro is exposed to medium (Spalevi *et al*, 2013, 2011, 2001) to excessive erosion, with highest values attained in the river catchments of Ibar and Piva and the coastal catchments (Spalevi *et al*, 2008, Kostadinov *et al.*, 2006, Lazarevi , 1996).

### **Geomorphological mapping methods**

Geomorphological mapping is essential for us to be able to understand the landforms and their genesis. Pavlopoulos *et al.* (2009) explain why mapping of landforms can be very helpful. Probably the most important reason is that mapping gets you a precise visualization of the region, which helps to fully determine the underlying processes and formation history. Furthermore, landforms can be connected and compared to each other (Pavlopoulos *et al.*, 2009).

Naturally, the evolution of Geographic Information System (GIS) technology extends the possibilities to visualize and analyse landforms, using GPS and artificial intelligence (Bishop *et al.*, 2012). An advantage of the development of these techniques is that it allows a part of geomorphologic mapping to a certain level without being forced to visit or stay in the study area. As Poppe (2012) indicated, for recognizing landforms and patterns, free high-resolution

(until 30m) ASTER Digital Elevation Models can be downloaded and analysed using GIS-software and Google Earth can be used as well, albeit for small to medium scale.

### **Research objectives**

From the beginning of the 20<sup>th</sup> century, plenty of research has been done about the landforms in Montenegro. However, writing this literature review, it became clear that not all the landforms are described and explained adequately. An attempt will be made to answer the research question (1) “*How did the geomorphology of Montenegro develop and what are its main features?*” Some answers were already given in the literature review. However, knowing that mapping is quite essential to determine underlying processes of landscape formation (see before), this will be the main part of the fieldwork.

First of all, it will be very useful to map Montenegro as a whole, with the different geomorphological regions and landforms, the main hydrography and the relief all put together in a clear, obvious way. A lot of geomorphological features have already been observed in detail by other authors, but too often the larger picture is missing, increasing the need for an overview. Creating this map will create a new overview of the geomorphology of Montenegro. For this map, the ‘geomorphon’ method (Jasiewicz and Stepinski, 2013) will be applied and compared to classic GIS-based methods.

The next objective of this research will be the mapping of some of the mentioned landforms in detail. For each landform a typical example is chosen using Google Earth and literature.

Landform types and/or locations of which a geomorphological map already exists are excluded. Consequently, glacial valleys of Durmitor (Hughes et al., 2011; Djurovic, 2009) and Prokletije (Milivojevic, 2008) will not be taken into account. Additionally, landforms that are smaller than 1 meter (such as karren) will not be mapped either. Njegusi is chosen as research area for a polje and Brca kanjon as a typical example of a canyon.

As written before, Montenegro is subject to intensive water erosion and land abandonment is identified as an important aspect, either favouring or countering land degradation. An answer on the following research question will be sought (2): “*What is the influence of land use changes on gully development?*” Based on the findings of Jeroen Van den Branden (2010), a few locations that satisfy these conditions will be defined. Other potential sites, including repeat photographs, have been preselected by promoters Prof. Dr. Nyssen and Dr. Spalevi . The results of this assessment will be mapped as well.

This part of the fieldwork will be executed in collaboration with Annelies Kerckhof, a fellow master student of the Department of Geography of Ghent University handling the interaction between humans and physical geography based on interview.

### **Materials and method**

For the small-scale map (overview) including the complete territory of Montenegro, the most important task will be to assemble all the existing literature and maps and put them together in one uniform map, using the legend proposed by Pavlopoulos *et al.* (2009). As geomorphologic information about the northern crystalline hills is quite scarce, it is necessary to do fieldwork there. A GPS will be used to determine the size and location of the major landforms. After the fieldwork GIS-software and Google Earth will be used to assemble and optimize these data in one map.

The detailed mapping of the smaller landforms and Skadar Lake will also be executed with the use of GPS. An additional aspect here will be the research of soil particles, i.e. qualitative recognition of soil texture. Therefore, it will be necessary to take samples of certain sediments. These samples will afterwards be investigated in the lab in Ghent to acquire a

better knowledge about the geomorphological history that explains the formation of the landforms. This will be useful for defining the origin of the different sediments present in the polje and canyons. Moreover, to develop a theory of the exclusion of Skadar Lake from the sea by the Bojana cone and dune formation, it will be essential to establish this theory with appropriate sediments. Similar to the small-scale map, the legend of Pavlopoulos *et al.* will be used to represent the landforms with GIS-software. For this part, Google Earth will be more difficult to use, taking into account its precision is approximately 40 meters (Chang *et al.*, 2009). Furthermore, the use of a digital camera will be useful to be able to investigate certain small landforms again if necessary.

The fieldwork of gully development changes (reactivation of stabilization) due to land use changes will be carried out together with Annelies Kerckhof. Again, use of GPS will be needed; however more precise measuring instruments could be necessary. For this part, the use of photographs will be essential.

### Conclusion

A variety of different processes in the past resulted in the formation of the present-day geomorphology of Montenegro. Since the end of the 19<sup>th</sup> century, researchers have been trying to explain the remarkable landforms. In this review, this research has been summarized to serve as a background and as a guide to the fieldwork that is performed in 2013 in the context of the master thesis: ‘Geomorphological mapping of Montenegro: landform genesis and current processes’.

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## ENVIRONMENTAL AND ECONOMIC EFFECTS OF FUEL ADDITIVES

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### Abstract

Today's public transportation still actively uses buses with outdated diesel engines that require significant investment for renovation and upgrading to facilitate modern filters. Possible solution is found in using additives based on saponified naphthenic acids, aliphatic hydrocarbons and copper. Utilizing these additives accelerates the engine's combustion process, reducing carbon and other exhaust gasses emission, carbon monoxide and particulate matter as well as fuel consumption, resulting in increased fuel economy.

Author's primary goal was to determine effects of additive utilization on fossil fuel combustion, emission of exhaust gases along with possible effects on fuel economy. Research was conducted in two cycles on public transport buses, one of an older type and one of the newer type. Data on fuel consumption was collected without additives for the first cycle and with fuel additives on the second one.

**Keywords:** environmental-economic effects, catalysts (additives), exhaust gases, savings, frugality

### Introduction

In recent years, the energy crisis has steered liquid fuel consumers towards a critical analysis of the fuel combustion process and discovering means for its reduction. An entire array of problems that affect the efficiency of the entire combustion process is evident, therefore the fuel consumption as well. Problems are mainly caused by (non) quality of the oil derivatives (fuel oil, heavy, medium, light and extra light). The presence of mineral impurities and moisture reduces the fuel caloric value thus impeding its preparation and combustion.

It is a fact that the liquid fuel tanks over time accumulate mud and water that are always present within the fuel. Sludge is composed of combustible and non-combustible components. Combustible organic components can be converted into fuel with the use of additives, while non-flammable components remain deposited on the bottom of the tank and must be cleaned mechanically.

In parallel, work was been invested in the development of combustion additives and other means of reducing emissions, thereby protecting the environment from pollution. Today, diesel fuel additives are often added to improve the physical and chemical properties of fuels to meet the growing requirements as well as to improve the performance of the fuel in use. Using various additives must also take into account that they do not induce any damage (corrosive properties and being prone to forming sludge and deposits in vital parts of the engine).

Formation of sediments occurs if the compounds in the form of salts are added as additives, as they might interact between themselves. The result of these chemical reactions might produce insoluble precipitates in the fuel. When using additives in diesel fuels, solubility of certain additives in diesel fuels as well as their compatibility and possible chemical reactions have to

be taken into account. These occasional adverse effects, however, do not reduce the potential for the use of diesel fuel additives.

In this research the authors have describe the applications and effects in treating diesel fuels with F.O.E. 32 additive.

Using F.O.E. 32 as a combustion catalyst has proven it as a significant figure in the efforts for energy saving as will be further explained.

### **Problem definition**

Today's public transportation still actively uses buses with outdated diesel engines that require significant investment for renovation and upgrading to facilitate modern filters.

Possible solution is found in utilizing additives based on metal-organic compounds, solvents and spray system dispersant agents. Additive F.O.E. 32 was developed by Vestal Chemed Corporation, Manchester Avenue Missouri, USA<sup>1</sup> for improving diesel fuel combustion. It is defined as a catalyst to accelerate the fuel combustion process with a significant effect in reducing the amount of air required for complete combustion of the fuel.

The use of additives reduces exhaust gas emission, as well as particulate matter and carbon monoxide, improves combustion and fuel efficiency. Rationale for utilizing fuel additives is found in the multiple effects it has on all stages of the combustion process - before, during and after combustion. Consumption amount of diesel fuel in the engine depends on several factors: fuel quality, the degree of engine functionality (aging), use patterns and season of the year.

Natural liquid fuel in its elemental analysis contains carbon (C), hydrogen (H), oxygen (O), sulfur (S) and mineral admixtures (ash and water). Mineral adulterants containing moisture reduce the content of combustibles and the fuel caloric value.

Fuel ignition temperature is very important for diesel engines, it should be lower than the temperature at the end of the compression cycle in the engine cylinder (550-600° C).

Engine workflow is also affected by the time from the fuel suction into the cylinder to its ignition - the induction period. Cetane number of diesel fuel amounts to 40-60 units and can be increased by 12-20 units if added fuel additives that enhance and accelerate its oxidation (burning).

At low cetane numbers the induction period of the engine lasts longer and leads to the in-cylinder piling up of fuel, its vapors and peroxides, which after ignition burn with a shockwaves.

Cetane number of the fuel affects the engine starting characteristics, smooth operation of the engine and the complete combustion as evidenced in reduced smoking and improved smoke quotient in the exhaust gases. The main effects of the use and benefits of additives FOE 32 can be classified into three groups:

- a) **Economic effects** - savings in the consumption of liquid fuels of 2-6 % depending on the fuel quality.
- b) **Environmental effects** - by using additives, the quality of the exhaust gases is regulated and reduced below the allowed limits (CO<sub>2</sub>, SO<sub>2</sub>, VO<sub>x</sub>, etc.). Improving combustion is followed by quieter engine operation, lowered residue and corrosion development at the head of the piston and exhaust valve; that produce pressure loss and lower power on the cylinder. Preventing the corrosion on the pump and the fuel intake ensures longer engine life while decreasing negative environmental effects.

1. Vestal Chemed Corporation, Missouri, USA

- c) **Marketing effects** – advent of international competition, increased environmental awareness and media interest in environmental issues are a factor influencing the

domestic producers of liquid fuels, forcing them to differentiate themselves from the competition and perform better in the market. The use of modern additives in liquid fuels is a strong marketing advantage that might be used in order to improve the company position on the market.

**Additive F.O.E. 32 contains:**

- Agents and dispersants that allow a uniform distribution of additives to all parts of the storage tank.
- Solvents of asphalt compounds, which tend to sediment on the bottom of the tank during mixing of incompatible fuels.  
Application of additives prevents settling and creates conditions for moving homogenous and uniform fluid which enables significantly more uniform combustion.
- Dispersing agents that develop water–oil emulsification effect and have the property to disperse water residue in a uniform manner in the form of microscopic particles that can be easily combusted without affecting the controlled combustion process.
- Copper based additive F.O.E. 32 has several advantages over other additives. It is an excellent emulsifier, meaning that it dissolves organic particles that remain dispersed in the fuel, not allowing them to deposit as sludge at the bottom of the tank.

The environmental benefits character of using additives should not be ignored, especially regarding the necessity of standardization of energy production business activities. Quality Management System (ISO 9000), environment management system and preventive actions to protect the environment (ISO 14000) are adopted to ensure efficient operations, particularly in the aspect of air quality management, municipal and hazardous waste management and preservation of water quality.

As the laws on the protection of the environment constrain the allowed amount of particles that may be emitted into the atmosphere, this incentivizes the industry to further reduce the carbon residue in the flue gases. Combustion catalysts can reduce carbon emissions by improving the efficiency of the combustion process.

Energy conservation and environmental protection are obviously of great importance. However, the use of catalytic combustion can bring additional benefits.

In order to further improve the effects resulting from use of additives FOE 32, additives are produced in different composition for each type of fuel. It is added to the central tank, transport tank and the consumer's tank. The most rational solution is mixing it in the central tank or cistern after loading.

Financial effects are achieved by the savings in fuel exploitation treated with an additive. For example, gasoline savings are min. 1,5 - 2%, 3-5% for diesel, fuel oil 3-5%, heating oil 3-6%.

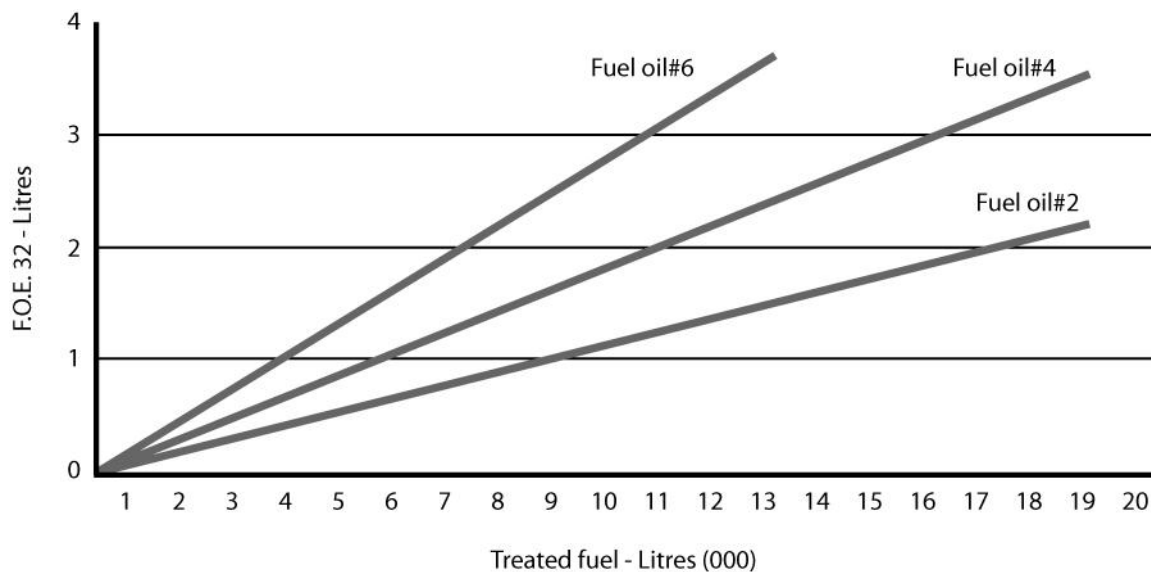
**The advantages of using F.O.E 32 additive:**

- ✓ Improved combustion
- ✓ Reduced fuel costs
- ✓ Elimination of tank corrosion
- ✓ Reduced cleaning costs
- ✓ Increase of the tank capacity
- ✓ Easy to use
- ✓ Positive for ecology
- ✓ Very economical

In recent years the energy crisis has forced company managements to a critical review of all aspects of the work, in order to determine the possibilities for the energy consumption



reduction. As an example, in 1974 the American Institute of paper launched the voluntary action program to improve energy efficiency in the industry. The main task was to reduce energy consumption by 10% by 1980. In June of 1976, the paper industry has achieved 10.7% reduction in fossil fuel use and fuel per unit of production compared to the first six months of 1972. At the same time, the use of self-generated and waste fuel increased by 3.9% per unit of output, so that the total energy used in manufacturing declined by 4.6% over the five year period. Despite this success, there is clearly a large number of steps to save energy that are still to be taken.



Graph 1. Additivition Vestal F.O.E. 32 combustion catalyst

### Materials and methods

Authors' method is based on the application of catalyst FOE 32 as follows:

Selected are two buses with different years of construction, bus no. 749, made in 1998. and bus no. 788, made in 2003. During the research, fuel consumption was measured in each bus during ten working days as well as the route traveled. At the end of the cycle the average fuel consumption was calculated. Exhaust emissions were monitored and recorded during the research.

The next test cycle consisted of the following steps:

The same buses were used with the addition of the F.O.E. 32 catalyst, proportional to the amount of fuel in the tanks. Monitoring of the fuel consumption and distance have continued. Two days of monitoring were selected when the buses were subjected to emissions recording. After the next ten working days period, the average fuel consumption has been calculated again.

Technical specification of the F.O.E. 32 catalyst:

Ingredients:

- 6% Cu-complexone
- 39% saponified naphthenic acids
- 55% aliphatic hydrocarbons

Appearance:

Clear liquid of blue-green color.

Dosage:

The optimal dose is 5 litres of F.O.E. 32 on 25 000 litres of fuel.

If the fuel is heavier in impurities, that is, if the fuel has more than the average amount of impurities to be combusted, a higher dose of the catalyst can be recommended.

The recommended dose should be added to the storage tank before the fuel is tanked in it. This method enables better mixing of the catalyst with fuel.

### Results and Discussion

Research was conducted on two test buses Volvo B10 M, garage numbers 749 and 788, JGSP Novi Sad. Additive treatment was done over a period of 11–25<sup>th</sup> of November 2005.

Table 1.

<b>BUS 749</b>	No additive treatment	With additive treatment	
Period	29.10. – 10.11.2005.	11.11. – 25.11.2005.	
Distance (km)	3.276	3.499	
Fuel consumption (lit.)	1.688	1.494	
Average fuel consumption (lit./100 km)	50,9	46,7	
Smoke quotient	0,608	0,578	0,466

Table 2.

<b>BUS 788</b>	No additive treatment	With additive treatment	
Period	29.10. – 10.11.2005.	11.11. – 25.11.2005.	
Distance (km)	3.133	3.065	
Fuel consumption (lit.)	1.494	1.436	
Average fuel consumption (lit./100 km)	47,7	46,9	
Smoke quotient	0,023	0,023	0,023

Survey results indicate:

- Fuel consumption of both observed buses was reduced using the F.O.E. 32 catalyst - by 8.25% on the bus 749 and by 1.7% on the bus 788.
- Exhaust gases were significantly reduced for the bus 749 and for the bus 788 exhaust gases remained at the same level.
- In both cases the exhaust emissions were below the allowed limits of the smoke quotient, which was the result of a more complete fuel combustion.

### Conclusion

- Conclusion of this paper is that there is great advantage of additive use in the period before combustion, during and after combustion process, according to the given results.

- The most important effect of catalyst use is ecological effect, because using a catalyst improves combustion, regulates emissions, prevents formation of deposits and corrosion.
- As for the financial impact, there is a significant saving for the diesel fuel in about 2-6 %.
- Catalyst can be applied in all liquid fuels. Due to its effect on the reduction of emissions, reduced fuel consumption it also has an economic justification so it remains highly recommended to be utilized for fuel treatment.
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Vestal Chemed Corporation, Missouri, USA

Directive 2003/17/EC assumes sulphur content of 10 mg / kg of gasoline and diesel fuel

Directive 2003/17/EC of the European Parliament and of the Council of 3 March 2003 amending Directive 98/70/EC relating to the quality of petrol and diesel fuels (1)

Directive 2009/40/EC of 6 May 2009 of the European Parliament and of the Council on roadworthiness tests for motor vehicles and their trailers, Official Journal L 141

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**THE WEIGHT STRUCTURE OF *PHOXINELLUS PSEUDALEPIDOTUS*  
(CYPRINIDAE) FROM THE MOSTARSKO BLATO (NERETVA RIVER BASIN,  
BOSNIA AND HERZEGOVINA)**

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**Abstract**

In this paper, the weight structure of endemic fish species *Phoxinellus pseudalepidotus* from the Mostarsko blato (Neretva River basin, Bosnia and Herzegovina) is presented. Specimens were collected monthly from January to December 2009 by gill nets (7 m length and 0.7 m height, with a 7 mm mesh size). The minimum recorded body weight was 0.1 g and maximum recorded weight was 15.0 g. The average of the total weight for the population was  $3.1 \pm 1.2$  g. Maximum and minimum weight was recorded for females. The average weight for females was  $3.3 \pm 1.4$  and for males  $2.9 \pm 0.9$ , and the range between the minimum and maximum values was higher in females (0.1 to 15.0 g) than in males (0.2 to 8.0 g). It was found that females had a higher body weight than males.

**Key words:** endemic species, *Phoxinellus pseudalepidotus*, weight structure, Mostarsko blato

**Introduction**

There have been recorded 40 endemic species, mostly native to particular localities, in the waters of BiH so far. The species richness, especially endemic species, classified Bosnia and Herzegovina in the group of the ichthyologically most diverse European countries. This can be attributed primarily to geographic location and isolation from other European river systems, as well as to complex geological history and characteristics of climate (Glamuzina et al., 2010).

*Phoxinellus pseudalepidotus* (Bogutskaya & Zupan i , 2003) is an endemic species that inhabits the Mostarsko blato, and is considered to be present in the wider area of the Neretva river basin (Bogutskaya & Zupan i , 2003). In the earlier literature (Heckel & Kner, 1858; Seeley, 1886; Kolombatovi , 1886; Trgov evi , 1905; ur i , 1913; Vukovi & Ivanovi , 1971; Vukovi , 1977b; Povž et al., 1990) the species was identified as *Phoxinellus alepidotus*, which is morphologically quite similar (Bogutskaya & Zupan i , 2003).

There are limited quantitative information available on the life history of *Phoxinellus pseudalepidotus*. This species inhabits shallow channels with little current and clean water (Bogutskaya & Zupan i , 2003). Like all other *Phoxinellus* species, this species withdraws during unfavorable periods to the groundwater, as well. The sex ratio of the population of *Phoxinellus pseudallepidotus* in the area of Mostarsko blato was 1:1.185 in the favour to females (Markoti et al., 2012). It is a relatively small cyprinid whose total lengths (TL) ranged from 2.7 to 11.5 cm (mean  $6.4 \pm 0.8$  cm). Female size ranged between 2.7-11.5 cm (mean  $6.5 \pm 0.9$ ), while in the males from 3.3 to 8.5 cm (mean  $6.4 \pm 0.6$  cm) (Markoti et al., 2013). The smallest ripe female measures 5.11 cm SL, and the smallest ripe male 4.87 cm SL (Bogutskaya & Zupan i , 2003). Seasonal diet of *Phoxinellus pseudalepidotus* consisted

mainly of insects, but gastropods, plant material and amorphous mass were also included (Markoti et al., 2013).

IUCN Red List Status of *Phoxinellus pseudalepidotus* is Vulnerable (VU D2) (IUCN, 2013).

### Materials and methods

Mostarsko blato area, through which the river Lištica flows, is the closed karst field in western Herzegovina. It is surrounded by limestone hills: Orlovac, Mikulja a, Vira a, Trtla and Varda. The field is flooded on average 5-6 months during the year. In addition to permanent water sources in Mostarsko blato, significant amount of periodic water of rivers Ugrova a, Orovnik, Mokašnica and a number of small flows, are active during the major rainfall seasons. In order to reduce the floods, the tunnel Varda was built in the year 1947. Except for the Varda tunnel, water from Mostarsko blato is managed also by the following sink holes: Krenica, Košina, Renkova a, Kruševo, and the Great Hole (Velika jama). These sink holes are important for *Phoxinellus pseudalepidotus* life cycle (Bogut et al., 2007).

The study was conducted at three stations in the area of Mostarsko blato from January to December 2009: Station 1 (Pisak, 43°19'52.9"N; 17°41'08.4"E), Station 2 (Me uri , 43°20'04"N; 17°41'04.3"E) and Station 3 (Pološki gaz, 43°20'36.2"N; 17°41'36.4"E) (Figure 1). Specimens were collected monthly (100 specimens) by gill nets (7 m length and 0.7 m height, with a 7 mm mesh size), and total of 1200 specimens of *P. pseudalepidotus* was analyzed. The weight of specimens was measured at the technical scale "Sartorius" type PT 1200 with an accuracy of 0.1 g. Given that this is an endemic species, most of the specimens were taken back into the water after the weight measurements.

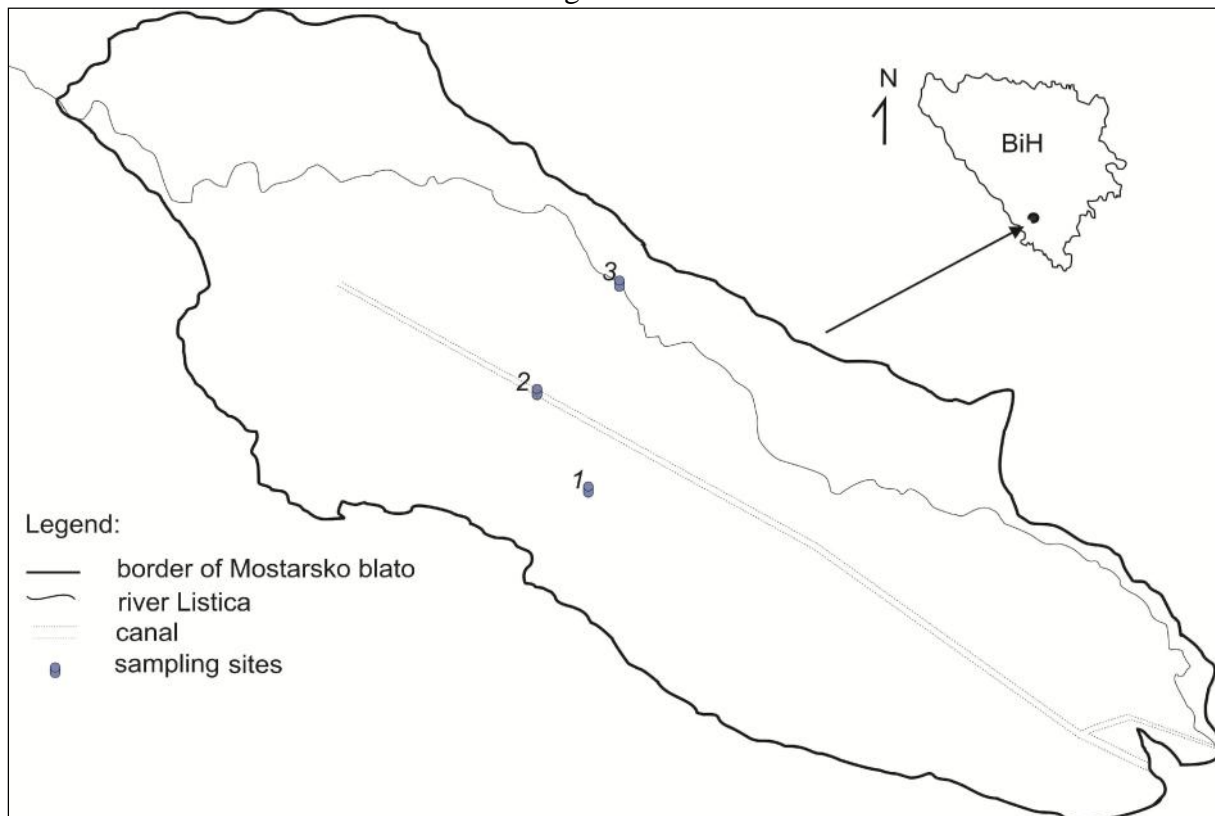


Figure 1. Map of sampling sites: Station 1 (Pisak), Station 2 (Me uri ) and Station 3 (Pološki gaz)

### Results and Discussion

All collected specimens of *Phoxinellus pseudalepidotus* were divided by the weight of the body into 13 classes. The classes within the population studied were formed 1.0 g body weight each, separately for the whole population, and for males and for females, respectively. Figure 2 shows that the predominant class is the one from 2.0 to 3.0 g weight with 492 specimens out of 1200, representing 41% of the population. The lowest prevalence was observed in the classes weighting from 9.0 to 10.0 g, than 11.0 to 12.0 g, and 12.0 to 13.0 g with just one specimen each.

The minimum recorded body weight is 0.1 g, and maximum recorded weight is 15.0 g. The average of the total weight for the population is  $3.1 \pm 1.2$  g. Maximum and minimum weight was recorded for females. The average weight for females was  $3.3 \pm 1.4$ , and for males  $2.9 \pm 0.9$ , and the range between the minimum and maximum values was higher in females (0.1 to 15.0 g) than in males (0.2 to 8.0 g).

The histogram of female weight (Figure 3) shows that females are also found in thirteen classes. The most prevalence was observed in the class from 3.0 to 4.0 g with 234 out of 651 specimens, representing 35.94 % of all the females. The following class is the one from 2.0 to 3.0 g consisted of 198 specimens, representing 30.41% of the total female sample. Males were distributed into eight classes. The histogram of male weight (Figure 4) shows that the most prevalence class is the one from 2.0 to 3.0 g with 294 out of 549 specimens, representing 53.55 % of all the males.

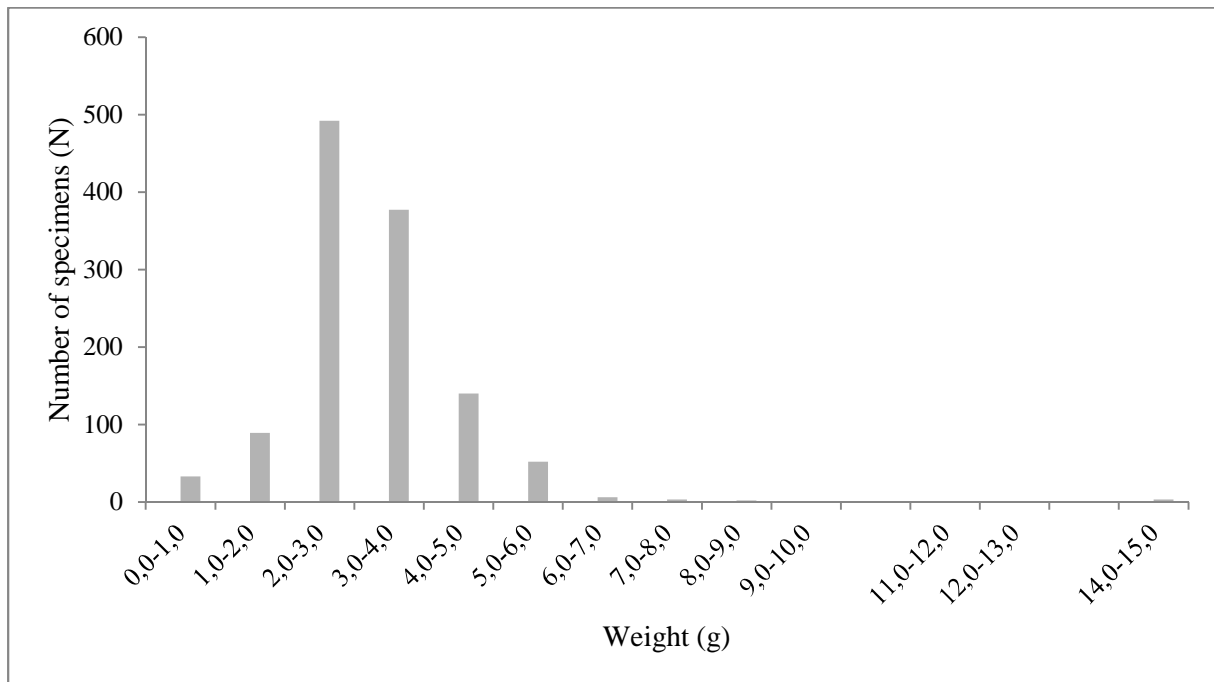


Figure 2. The weight structure of population of *Phoxinellus pseudalepidotus* in the Mostarsko blato

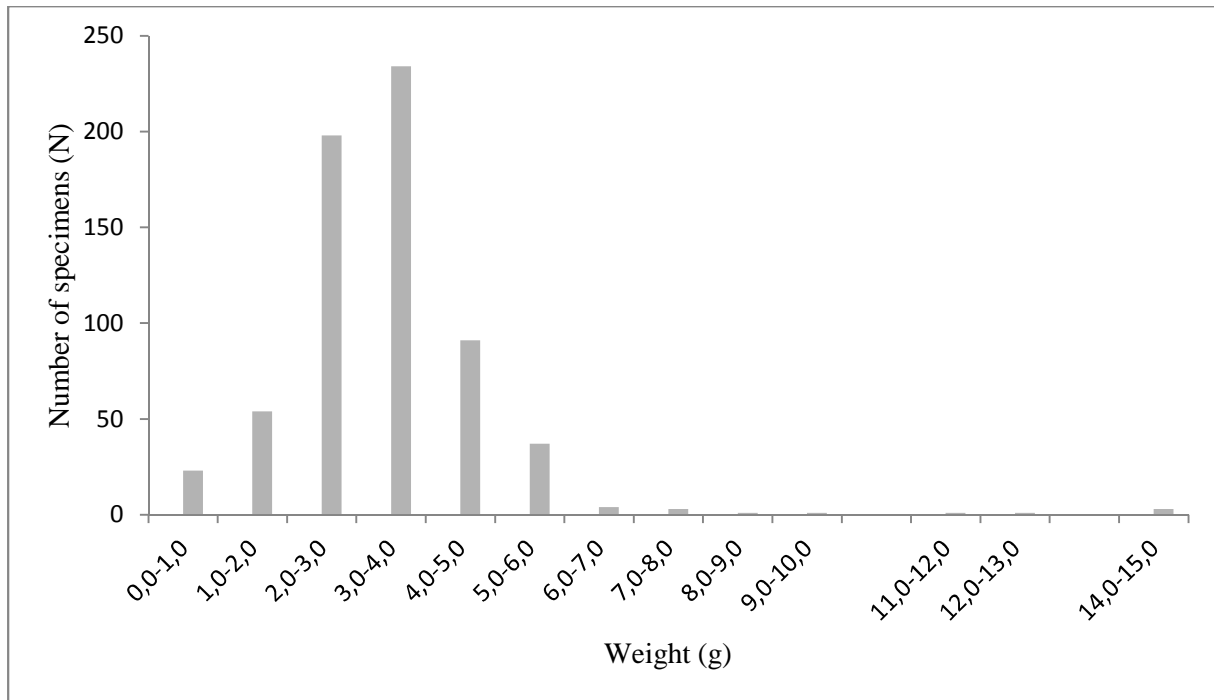


Figure 3. The weight structure of females of *Phoxinellus pseudalepidotus* in the Mostarsko blato

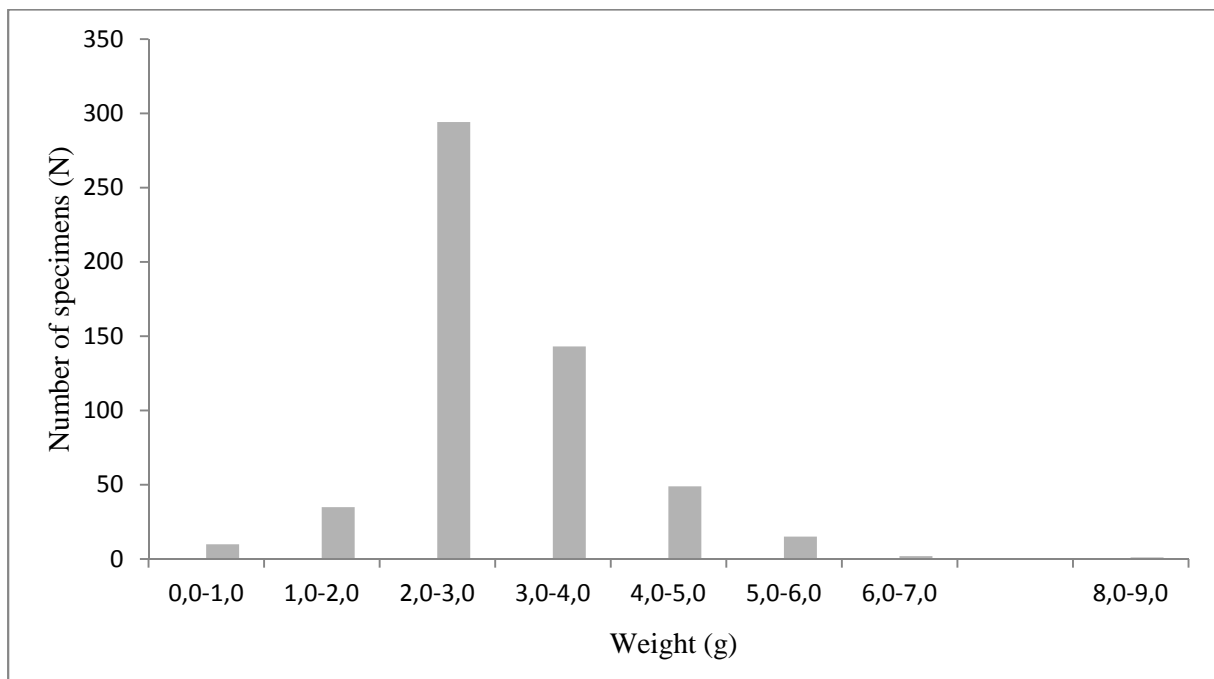


Figure 4. The weight structure of males of *Phoxinellus pseudalepidotus* in the Mostarsko blato

Females of *Telestes ukliwa*, endemic to the Cetina River in southern Croatia, ranged in weight from 3.2 to 28.1 g, and males from 1.3 to 25 g. Females of this species have a higher weight than males (Zanella et al., 2009). The minimum and the maximum recorded weight of

*Telestes ukliva* are higher than the minimum and the maximum weight of *Phoxinellus pseudalepidotus*. It was also found that females of *Telestes montenegrinus* in the delta of Skadar Lake have a higher weight than males (Krivokapi , 2002b). Frani evi and Ti ina (2003) reported that the weight of the analyzed specimens of *Delminichthys adspersus*, widespread in Red Lake in Imotski, ranged from 4.36 to 18.79 g. The minimum and the maximum recorded weight of *Delminichthys adspersus* are also higher than the minimum and the maximum weight of *Phoxinellus pseudalepidotus*. The average of the total weight of *Delminichthys adspersus* was  $12.51 \pm 1.51$  g. *Telestes metohiensis* from Bile a Lake in Bosnia and Herzegovina ranged in weight from 16.1 to 41.4 g, and the average of the total weight for this species was 27.7 g (Haskovi et al., 2007).

### Conclusion

The results of this study show that the endemic fish species *Phoxinellus pseudalepidotus* from the Mostarsko blato ranged in weight from 0.1 to 15 g. The average of the total weight for the population is  $3.1 \pm 1.2$  g. Maximum and minimum weight was recorded for females. The average weight for females was  $3.3 \pm 1.4$ , and for males  $2.9 \pm 0.9$ , while the range between the minimum and maximum values was higher in females (0.1 to 15.0 g) than in males (0.2 to 8.0 g). It was found that females had a higher body weight than males.

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**COCCIDIOSTATS IN POULTRY MANURE – A POTENTIAL THREAT TO  
BENEFICIAL SOIL INVERTEBRATES**

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**Abstract**

Monensin and lasalocid are polyether ionophore antibiotics used in veterinary medicine for prevention and treatment of coccidiosis in poultry. They are extensively used in the poultry industry throughout Europe. Both substances are excreted with the faeces mostly in their active form. The use of manure from treated animals on agricultural soil results in contamination that could pose a threat to soil organisms and thereby reduce the production potential of farmlands. Their degradation rates in manure and soil, as well as their effects on non-target soil organisms are mostly unknown. We conducted several studies to measure the degradation of lasalocid and monensin in broiler manure and after application to soil. Ecotoxicological studies were also performed to obtain concentrations at which these coccidiostats are harmful to soil invertebrates, namely earthworms and woodlice. Degradation rates in manure and compost depend mostly on moisture levels and temperature. Half-lives in compost are significantly shorter than if manure is aged in a pile with no treatment. Avoidance of the test animals was the most sensitive endpoint in the ecotoxicity tests. On the basis of our results, we recommend that poultry manure from treated animals be stored for at least one month before application to soil.

**Keywords:** coccidiostats, lasalocid, monensin, manure, agricultural soil

**Introduction**

The growing needs of the human population drive modern agriculture into using increasing amounts of pesticides and fertilisers, thereby increasing the threat of contamination to soils and underground water. Intensive use of manure burdens the environment not only with large amounts of nitrogen and metals, but also with veterinary pharmaceuticals and feed additives. Their introduction into the environment is not controlled, a problem that has been neglected for decades (Boxall, 2004).

Coccidiosis is a protozoal infection in poultry causing diarrhoea and dysentery. It is often fatal and spreads rapidly. Coccidiostats are authorised in the European Union as feed additives for poultry. Overall in the EU, of the estimated 40.65 million tonnes of feed produced, some 18.33 million tonnes is manufactured with an in-feed coccidiostat (EC, 2008). Broilers and turkeys are treated with coccidiostats almost their entire life. The most frequently used coccidiostats, monensin and lasalocid are natural ionophore antibiotics produced by bacteria of the genus *Streptomyces*. In treated animals these substances are only partially metabolised

and are thus excreted predominantly in the active form. EFSA (2004) reported that 74–83% of lasalocid in broiler excreta is in the active form and more than 50% of the excreted monensin is the parent compound (Davison, 1984).

The degradation rates of monensin in manure range between 3 and 21 days (EFSA, 2004; 2005; Dolliver et al., 2008) and the rate of lasalocid degradation is mostly unknown. When in the environment, the coccidiostats undergo both biotic and abiotic degradation, with microbial degradation being the prevalent (Vertesy et al., 1987; Sassman and Lee, 2007; Hansen et al., 2009b; Hansen et al., 2012). The rate of decay depends on the organic content of the soil, soil moisture, temperature and pH (Sassman and Lee, 2007; Yoshida et al., 2010), but it has so far only been investigated under laboratory conditions. The reported monensin half-lives in soil are between 2 days (Sassman and Lee, 2007) and 22.7 days (Yoshida et al., 2010). EFSA (2004) reports lasalocid half-life values of 0.6–14.2 days. The reports on the predicted environmental concentrations (PEC) of monensin vary greatly and are in the range of 0.05–1.12 mg kg<sup>-1</sup> soil (EFSA, 2005; Žižek et al., 2011). The PEC for lasalocid is estimated at 0.58 mg kg<sup>-1</sup> soil (EFSA, 2004).

In spite of extensive use of monensin and lasalocid for more than 40 years and their potential presence in the environment, there is only little published information concerning their effects on non-target organisms and their fate in the environment. This lack of data was also found by (Hansen et al., 2009a), who stressed that a complete risk assessment cannot be performed without them. Several investigations into the risk of the two most common coccidiostats were therefore undertaken at the Laboratory for forensic toxicology and ecotoxicology at the Veterinary Faculty, University of Ljubljana. We studied their degradation in manure and soil and explored their effects on beneficial soil invertebrates.

## Materials and Methods

### Degradation experiments

Degradation of monensin in manure and compost has already been studied (Dolliver et al., 2008). In a similar way, an experiment was conducted with lasalocid. Chicken manure with no coccidiostats was obtained from a farm in Slovenia. It was divided in two piles, one of which was mixed with wood shavings to obtain a C/N ratio of approximately 30:1. Lasalocid was mixed into a sub-sample of both piles at the concentration 10.6 mg kg<sup>-1</sup> dry weight. Lasalocid-containing manure was put into nylon mesh bags containing approximately 30g of sample. The bags were put in the middle of the manure/compost pile. The experiments were performed in 1 m<sup>3</sup> polypropylene bioreactors with perforated bottoms and an inlet for aeration. Temperature was monitored throughout the experiment. Compost was constantly aerated and moisture was adjusted weekly to approximately 60%. Samples were taken at 2-day intervals for the first week and once a week thereafter. They were stored at -20°C until analyses. Lasalocid was measured with HPLC using the method described in Žižek and Zidar (2013).

For degradation in soil, lasalocid was mixed with the same manure to obtain a concentration of 3 mg kg<sup>-1</sup>. Manure was applied to soil at levels corresponding to 10, 20 and 30 tonnes per hectare. Samples were taken from five different locations on each plot and a cumulative sample was made at each sampling. Samplings took place at two-day intervals in the first week and in four-day intervals thereafter.

The half-life of lasalocid in manure, compost and soil was estimated using the Gustafson-Holden bi-phasic kinetic model  $C_t = C_0(1 + \gamma t)^{-1}$  where  $C_t$  and  $C_0$  are concentrations at time  $t$  and at the beginning, respectively, and  $\gamma$  and  $\lambda$  are the parameters of the gamma probability density function of the degradation constants. The half-life of lasalocid was calculated as  $(0.5 - (1/\lambda) - 1)/\gamma$  (Gustafson and Holden, 1990).

### Toxicity tests

Ecotoxicity tests were performed with both coccidiostats. The test animals used were earthworms (*Eisenia andrei*) and isopods (*Porcellio scaber*). We measured the effects of lasalocid and monensin on earthworm survival, growth and reproduction and on isopod survival, growth and food consumption. In earthworm acute and chronic toxicity testing we followed the protocol of OECD (OECD, 2004) and with isopods, the work was performed using the procedure described in Hornung et al. (1998). The performed tests are described in more detail in Žižek et al. (2011) and Žižek and Zidar (2013).

There has been indication that standard toxicity test may not be sufficiently sensitive for assessing the threat to soil organisms. As an alternative to standard ecotoxicity tests and a rapid and cost-effective first screening tool for soil assessment, avoidance behaviour tests have been proposed (Yeardley et al., 1996; Natal da Luz et al., 2004; Loureiro et al., 2005; Amorim et al., 2008). We therefore conducted avoidance tests with lasalocid on both test species. The procedure is described in detail in Žižek and Zidar (2013).

### Risk assessment

After obtaining the exposure levels with the degradation experiments and the effect concentrations with the toxicity tests, it was possible to use the newly obtained values to reassess the risk posed by monensin and lasalocid to agricultural ecosystems. This was done according to the EC Technical Guidance Document (EC, 2003). As the predicted no-effect concentrations (PNECs) we used the lowest values obtained by the toxicity tests. We formed recommendations regarding the most appropriate way and period of storing manure contaminated with coccidiostats before it is applied to agricultural soil.

## Results and Discussion

The results of lasalocid degradation experiments are presented in Figures 1 and 2. Lasalocid in manure degraded with a half-life of 61.8 days and did not fall below 45% of the initial concentrations even after 84 days, whereas its half-life in compost was 17.5 days and the concentrations fell below the limit of detection (10 ng g<sup>-1</sup>) after 80 days. The average half-life of lasalocid in soil was 3.1 days. Dissipation in soil includes wash-off with rain, as well as biotic and abiotic degradation.

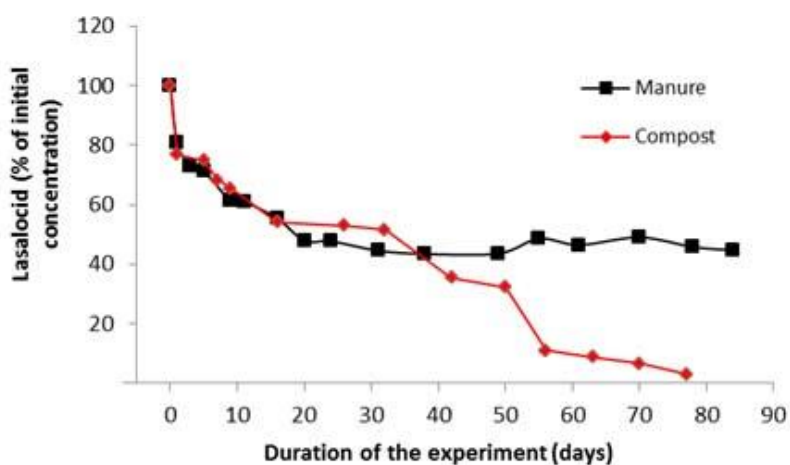


Figure 1: Degradation of lasalocid in manure and compost

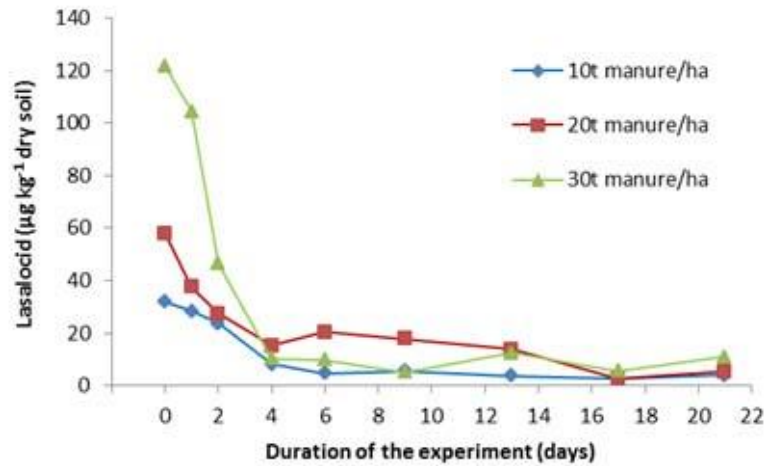


Figure 2: Degradation of lasalocid in soil

The results of the ecotoxicity tests are summarised in Table 1. In general, earthworms are more susceptible to both coccidiostats than isopods. This could be due to their thin integument and greater exposure to the environment. Avoidance test showed much higher sensitivity to ionophore antibiotics compared to the ‘classical’ sublethal toxicity tests. For earthworms, EC<sub>50</sub> for avoidance was more than five times lower than the EC<sub>50</sub> for reproduction. In isopods we recorded even higher sensitivity. While the highest lasalocid concentration had no significant effects on isopod growth or survival, already the lowest used concentration in the behavioural assay (4.51 mg kg<sup>-1</sup>) caused significant impact on isopod behaviour. Avoidance behaviour could indicate a potential adverse effect of lasalocid on the habitat function of soils where lasalocid-contaminated manure is applied.

 Table 1: Results of the toxicity tests (LC<sub>50</sub> – 50% lethal concentration, EC<sub>50</sub> – 50% effect concentration, NOEC – no observed effect concentration)

Test species	Test compound	Effect
<i>Eisenia andrei</i>	Monensin	LC <sub>50</sub> = 49.3 mg kg <sup>-1</sup>
		EC <sub>50</sub> reproduction = 12.7 mg kg <sup>-1</sup>
	Lasalocid	NOEC reproduction = 3.5 mg kg <sup>-1</sup>
		LC <sub>50</sub> = 156 mg kg <sup>-1</sup>
<i>Porcellio scaber</i>	Monensin	EC <sub>50</sub> reproduction = 69.6 mg kg <sup>-1</sup>
		NOEC reproduction = 4.77 mg kg <sup>-1</sup>
		EC <sub>50</sub> avoidance = 12.3 mg kg <sup>-1</sup>
	Lasalocid	LC <sub>50</sub> > 849 mg kg <sup>-1</sup>
		EC <sub>50</sub> growth > 849 mg kg <sup>-1</sup>
		NOEC growth > 849 mg kg <sup>-1</sup>
<i>Porcellio scaber</i>	Lasalocid	LC <sub>50</sub> > 277 mg kg <sup>-1</sup>
		EC <sub>50</sub> growth > 277 mg kg <sup>-1</sup>
	Lasalocid	NOEC growth > 277 mg kg <sup>-1</sup>
		EC <sub>50</sub> avoidance = 4.9 mg kg <sup>-1</sup>

By applying the obtained toxicity data in the risk assessment, we saw that using fresh manure contaminated with coccidiostats could pose a threat to soil invertebrates and thereby harm the production potential of agricultural soil. It is strongly recommended that manure should be aged for at least one month before application.

### Acknowledgement

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**VIRTUAL WATER BALANCE ESTIMATION IN AN IRRIGATED AREA IN  
NORTH-EASTERN TUNISIA**

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**Abstract**

The virtual water concept, defined by Allan (1997), as the amount of water needed to generate a product of both natural and artificial origin, this concept establish a similarity between product marketing and water trade. Virtual Water trade can alleviate, in arid countries, the problem of water scarcity, increasing imports of products with high virtual water content and so, it can allocate scarce resources to higher priority uses.

Given the influence of water in food production, virtual water studies focus generally on food products. At a global scale, the influence of these product's markets with water management was not seen. Influence has appreciated only by analyzing water-scarce countries, but at the detail level, should be increased, as most studies consider a country as a single geographical point, leading to considerable inaccuracies. For this reason, we consider the value of exploring virtual water strategy at smaller scales such as an irrigated area.

The main objective of this work is the estimation of virtual water balance of strategic irrigated crops (fruit trees and vegetables) in Semi-arid area in Tunisia to determine their influence on the water resources management and to establish patterns for improving it. The virtual water balance was performed basing on farmer's surveys, crop and meteorological data, irrigation management and regional statistics.

**Keywords:** *Virtual Water, North-Eastern Tunisia, Irrigated Crops, Water Management.*

**Introduction**

To confront water scarcity and support food security, the concept of virtual water is used. As defined by Allan (1997) virtual water is “the water embedded in key water-intensive commodities such as wheat” or “the water required for the production of commodities”. The importance of this concept is related to its potential contribution for saving water, especially in water short regions like Tunisia. This research study tries to evaluate the strategic importance of polluted or gray water (Loiseau, 2010), which is a component of virtual water with green and blue ones. Reduction of virtual water for strategic agricultural products can be obtained by reduction of gray water. The latter is defined as “water required to dilute polluted water to reach the normalized quality, different with countries”. Water pollution is especially related to use of chemical products (fertilizers, pesticides, etc.) for some crops like vegetables. Besides having a lower opportunity cost, the use of green water for the production of crops has generally less negative environmental externalities than the use of blue water (irrigation with water abstracted from ground or surface water systems). Tunisia exports some crops and gray water volumes in exports have rarely been estimated. Thus, estimation of gray water plays a role in ensuring water and water-dependent food security and avoiding further potential damage to the water environments in both importing and exporting countries.



In this context, Tunisian semi-arid region is chosen because of presence of dry and shiny period, occurring after a cold and rainy one, useful for vegetables crops and family food security. The aim of this study is to present methodologies which can be used to reduce virtual water for some strategic crops in Tunisian semi-arid region, based on improve of irrigation techniques and control of runoff and leaching water;

### Material and methods

To estimate the virtual water for different crops, several models were used with the objective to determine the water consumed by the plant. In this study, net irrigation requirements for studied crops and regions were computed following the FAO56 method (Allen et al, 1998) from meteorological data available.

Crop evapotranspiration (ETM, equation 1) was estimated from reference evapotranspiration (ET<sub>0</sub>) and the appropriate crop coefficients (K<sub>c</sub>).

$$ETM = K_c ET_0 \quad (1)$$

Reference evapotranspiration (ET<sub>0</sub>) was computed using the Penman-Monteith method (Smith 1993). The crop coefficients values at the initial, medium and end of the crop stages (K<sub>c ini</sub>, K<sub>c med</sub> and K<sub>c end</sub>), the general lengths (L) for the different growth stages (L<sub>ini</sub>, L<sub>dev</sub>, L<sub>mid</sub> and L<sub>late</sub>) and the total growing period for the main crops. Net Irrigation requirements (NIR, equation 2) were calculated using the standard FAO procedures, as described by Allen et al. (1998). Effective precipitation (EP) was calculated using the empirical USDA method (Cuenca, 1989). Following these procedures, reference evapotranspiration (ET<sub>0</sub>), crop coefficients (K<sub>c</sub>), crop evapotranspiration (ET<sub>c</sub>), effective precipitation (EP) and net irrigation requirements (NIR) were estimated for the main crops in the AID in 2011.

$$NIR = (K_c ET_0) - EP \quad (2)$$

Net irrigation requirements calculations are based on the soil moisture regime and the phenological stage of the crop, while keeping the other variables at the optimal production level. On this basis, we can calculate crop coefficients for a given location. It is also possible to construct the mathematical function that connects the crop water consumption to the desired crop yield.

The choice of a model depends on the objectives of the study. When the most important is the relationship between water and crop production, which is the case, FAO models (AQUACROP and CROPWAT) are frequently used. CROPWAT is the simplest, based on empirical relationships between water availability and production.

In this study, virtual water consumed by crops was calculated as green (water provided by rain) and blue (water provided by irrigation) water. The present study estimates the green and blue water footprint of 1 kilogram of studied crops produced in semi-arid area in Tunisia following the method described by Hoekstra et al. (2009).

In the study, vegetable production in the different Tunisian semi-arid regions was considered, distinguishing production throughout the year as well as between growing systems. The study focuses on the production stage, that is, the cultivation of the product, from sowing to harvest. The crop virtual water was calculated for each year distinguishing the green and blue water components.

The virtual water of studied crops (rainfed or irrigated) has been calculated distinguishing the green and blue water components Within the CROPWAT model (FAO, 2009)., the ‘irrigation schedule option’ was applied, which includes a dynamic soil water balance and keeps track of the soil moisture content over time. The calculations have been done using climate data from representative meteorological stations located in the major crop-producing regions, selected depending on data availability.

Low virtual water values can be obtained by use of green water and reduction of blue water, based on improve of irrigation techniques and control of runoff and leaching water. For Tunisian semi-arid region, the best seasons for this are spring and autumn.

Vegetable crops generally need a large amount of workers, which can bring agricultural income, especially for women and children and then target food security for them. To perform this, statistical and field analysis of workers (ONAGRI, 2010), for Tunisian semi-arid region, were used.

### **Results and discussions**

Figure 1 presents the net irrigation requirements (NIR) for the vegetables crops and irrigated zones in semi arid area.

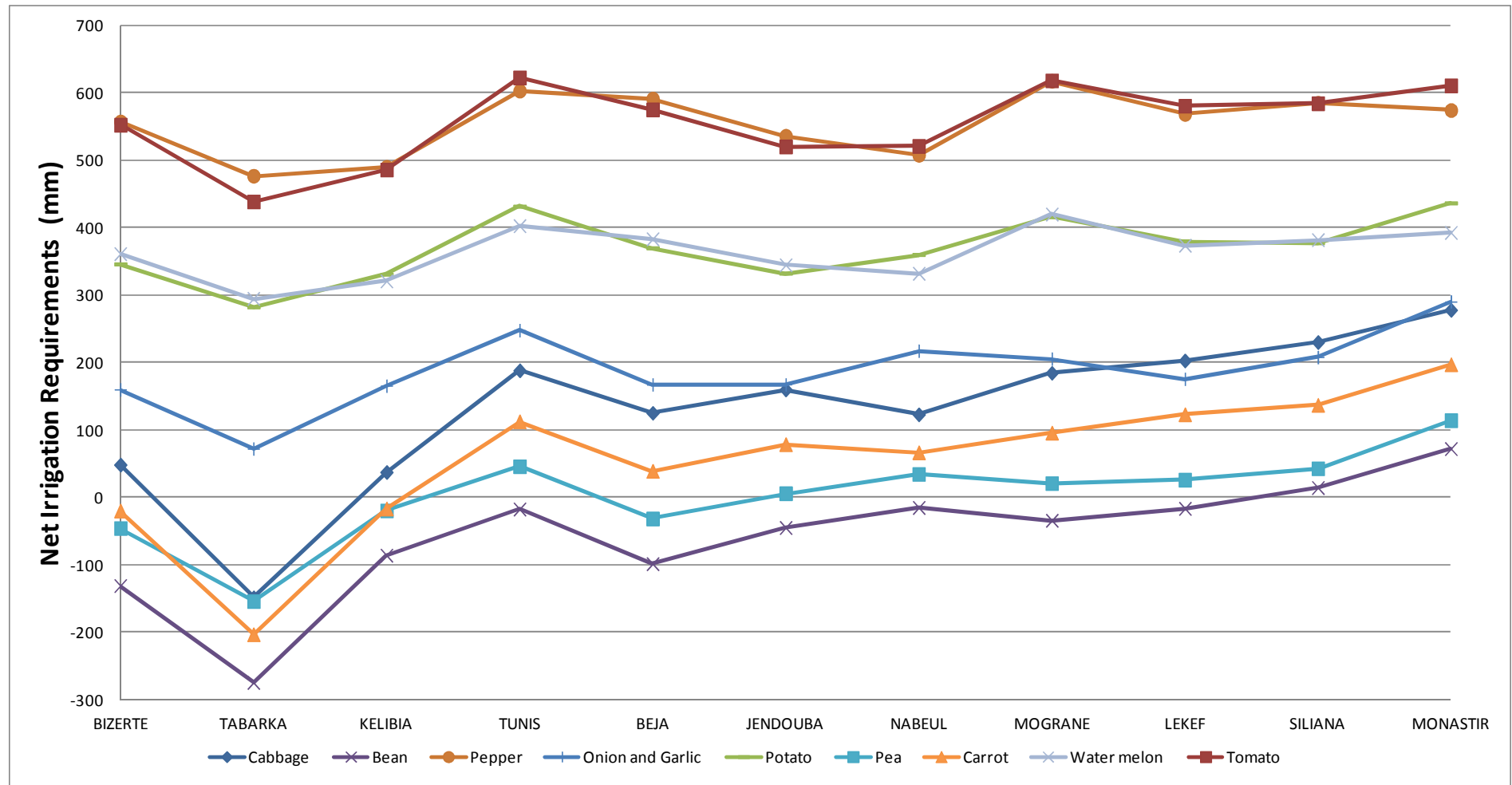


Figure 1. Net irrigation Requirements (mm) for studied vegetables in Tunisian semi arid areas.

NIR slightly increase for all studied crops from the north to the center of Tunisia, this increase is due to that central regions of the country receive less rain. Some vegetables such as pepper, pea and bean do not need irrigation in some regions and have a negative NIR, which means that virtual water consumed by these crops was only provided by the rain or green water. Cultivating these crops in these areas of the country helps minimize irrigation water consumption and thus the virtual water reduction. Summer crops such as tomato and pepper have the highest NIR from 430 mm in Bizerte to 600 mm in Monastir.

Figure 2 presents the net irrigation requirements (NIR) for fruit trees and irrigated zones in semi arid area.

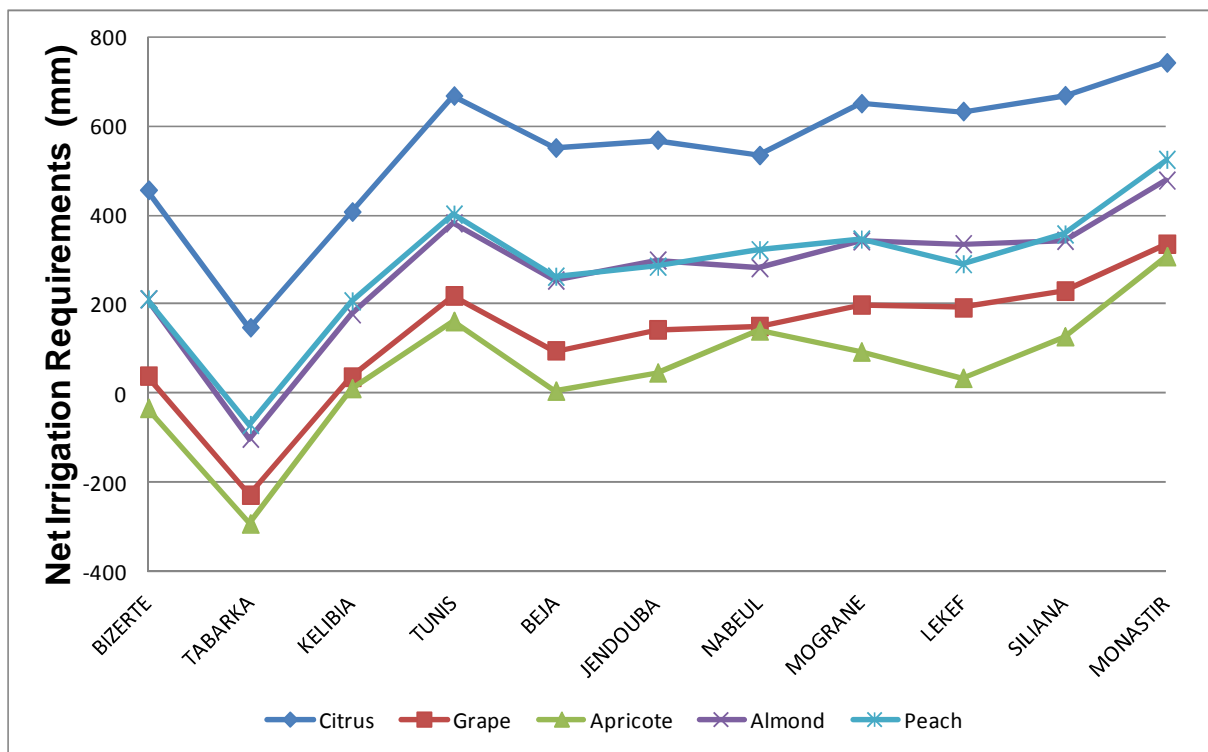


Figure 2. Net irrigation Requirements (mm) for studied fruit trees in Tunisian semi arid areas.

The NIR slightly increase for all studied crops from the north to the center of Tunisia, this increase is due to that central regions of the country receive less rain. Some fruit trees such as citrus, Apricote and Almond do not need irrigation in some regions and have a negative NIR, which means that virtual water consumed by these crops was only provided by the rain or green water. Cultivating these crops in these areas of the country helps minimize irrigation water consumption and thus the virtual water reduction. Summer crops such as citrus have the highest NIR from 180 mm in Tabarka to 780 mm in Monastir.

Awareness of the farmer to managing water resources is related to knowing the real crop water requirements at different stages of crop development although he manages well the irrigation scheduling. Over irrigation as infra-irrigation, have a negative impact on crop productivity. Finally, the virtual water concept, contextualized in space and time can provide useful information for benchmarking, identifying best practices and achieving a more integrated water resource management. Nevertheless, to obtain a comprehensive picture, not

only the (eco) efficiency in terms of m<sup>3</sup>/ton should be considered, but also the context-specific total cumulative virtual water.

### Conclusions

The use of the virtual water concept to confront water scarcity and support food security, in Tunisian semi-arid region showed that:

- Spring and autumn vegetable crops present low virtual water and are thus recommended for this region;
- vegetables are one of the most important agricultural activities in the country contributing to food security needs in water are relatively high compared to other agricultural products;
- Citrus present the high virtual water in the studied regions;
- Reduction of virtual water for these vegetables crops in Tunisian semi-arid region, can be obtained by improve of irrigation techniques and control of runoff and leaching water, using drip, localized and underground irrigations ;
- The virtual water estimation by using a model that gives enough information to perform the value of consumed water each crop and the water wasted by the farmer can help to guide agricultural policy for better water management;
- The concept of virtual water should be treated with caution in trying to both manage water resources according to speculation and ensure food security;
- It seems clear that integrated water allocation, planning and management is needed in the Tunisian semi-arid regions, considering the environmental water requirements together with the blue (surface and ground) and green virtual water, to achieve a more compatible agricultural production.

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## **5. ANIMAL HUSBANDRY**

**PASTORAL DYNAMICS IN THE REGION OF DIFFA (NIGER): A DESCRIPTIVE ANALYSIS OF LIVESTOCK CAPITAL**

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**Abstract**

Located between the desert zone in the North and the Sahelian zone in the South, the Region of Diffa is a pastoral area par excellence in Niger. Breeding, with a highly diversified livestock, is the dominant economic activity in the region. It occupied 95% of the population and contributes annually to 55% in the formation of the region gross domestic production (1). For understanding the pastoral dynamic a survey of 300 households (150 households with herd sedentary and 150 with herd mobile) was conducted during the first semester of 2012. The data analysis is performed from a herd of 15,618 animal heads consisting mainly of small ruminants for both sedentary (72%) and mobile (52%). The herd structure (sedentary and mobile) by age and sex shows on the one hand, the males are early and systematically exploited, all species including, and the reproduction is carried out by a core of female spawners more less stable and dominated by young females, on the other hand. Comparative analysis of compositions and structures of the livestock by agro-ecological zone (Pastoral bowls zone; Komadougou River and Lake Chad zones) reveals zonal disparities particularly in the sedentary livestock system.

**Keywords:** Livestock, Pastoral economy, livestock system, Diffa, Niger

**Introduction**

Animal husbandry is practiced throughout Niger territory according of agro-climatic parameters. This activity contributes significantly to household budgets and to meeting the food needs of Nigerien populations (Republic of Niger, 2003; Save the Children, 2009).

Livestock sector represents 70% of export agricultural products, 11% of the country's GDP. It is the second export heading (22% of export revenues) of the country after 'uranium (Republic of Niger, 2003). However, various natural constraints (rainfall, silting up of ranges, etc.) and anthropogenic (demographic pressure) seriously affect all pastoral production systems in Niger, especially in the Region of Diffa, an essentially pastoral zone with 10% of the national livestock (Republic of Niger, 2008b). In this Region, 95% of population practices animal husbandry as their primary or secondary economic activity after farming.

To understand livestock dynamics in the Region in the face of constraints which have become more and more structural over the years, 300 households were surveyed during the first semester of 2012. This article provides a descriptive analysis of the livestock surveyed with a sample of 15,618 head of animals. After a look into natural environment of the region, the composition and structure of livestock surveyed in general were analysed. In second hand, an

analysis by rearing system (sedentary or nomadic) and agro-ecological areas will be conducted in order to identify the major trends and potential variances that may exist.

### Natural environment

With a surface area of 156,906 Km<sup>2</sup>, the Region of Diffa is located in the far Eastern part of Niger between 10° 30' and 15°35' east longitude, 13°04' and 18°00' north latitude (Figure 1).

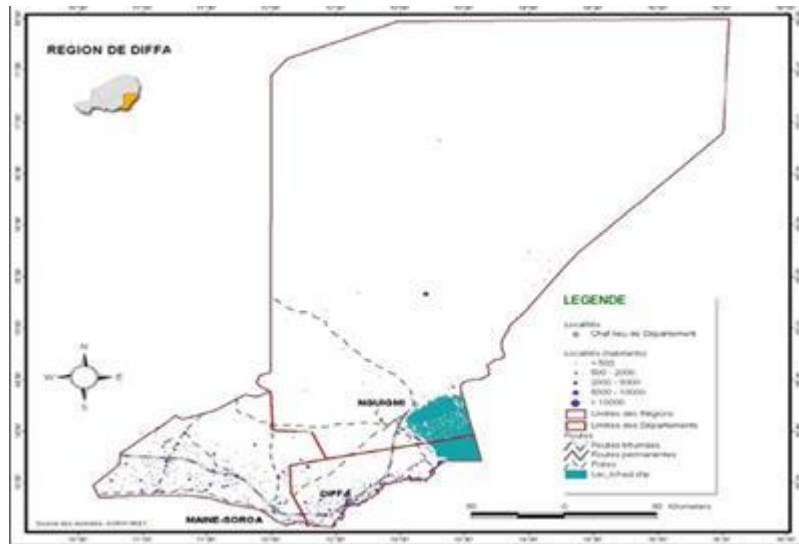


Figure 1: Spatial distribution of communities in the Region of Diffa (source : Republic of Niger, 2006).

The climate is Saharan-Sahelian. This makes the Region of Diffa one of the pastoral zones per excellence in Niger.

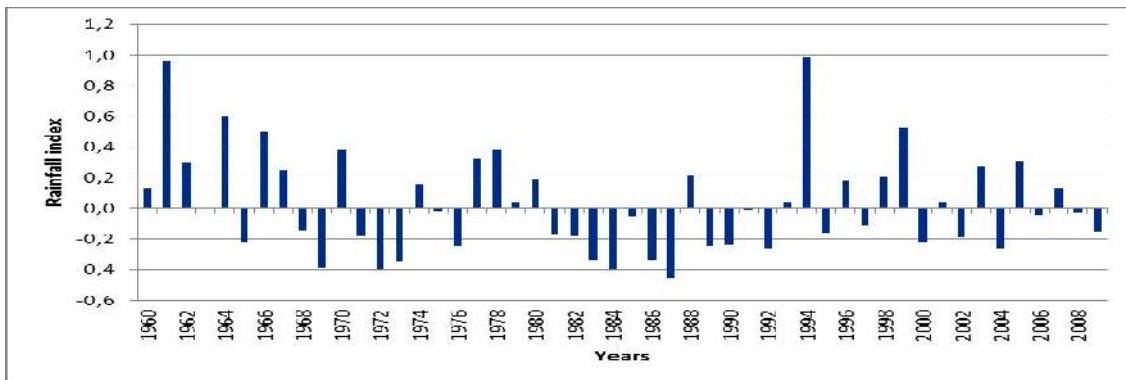


Figure 2. Rainfall variations in the Region of Diffa from 1960 to 2009  
Source : INS data (2010).

Figure 2 shows the evolution of rain index in the Region of Diffa from 1960 to 2009 characterized by a succession of more or less constant dry periods. Only two years out of 11 recorded excess rainfalls over the period 2000 - 2011. This climate variability generates



recurrent shocks and important fodder deficits for an extensive rearing system. Such deficits are becoming more and more structural (figure 3).

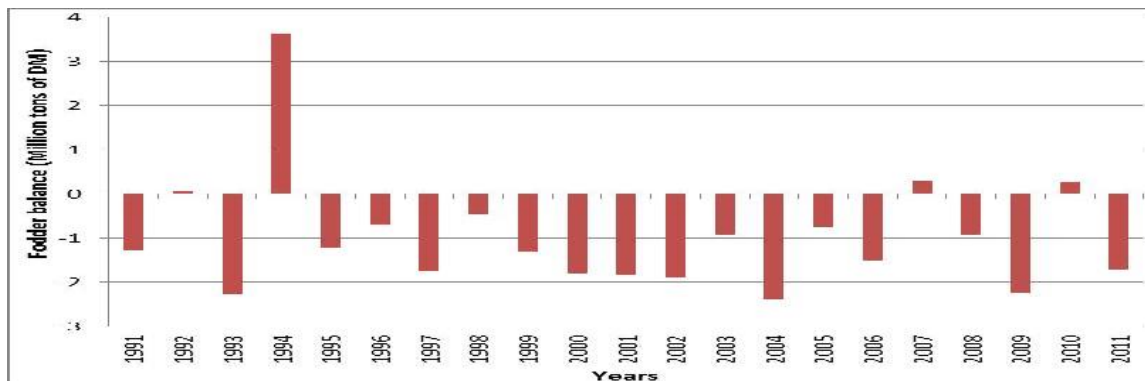


Figure 3. Evolution of fodder balances in the Region of Diffa from 1991 to 2011  
Source: Data from DNP (2010) and DREIA (2012)

### Materials and methodology

Based on agro ecological parameters, the Region of Diffa has been divided into three (3) survey areas (pastoral bowls zone, Lake Chad zone and Komadougou River zone).

#### Agro ecological zoning

- Pastoral bowls zone: It corresponds to the Sahelo-saharan belt laying between isohyets 150 and 250 mm per annum (Northern part of the Region of Diffa). Livestock is the main economic activity of communities (Fulanis, Mangas, Arabs and Toubous).
- Komadugu zone: It is located in the Southern part of the Region, 150 km along Komadugu Yobe River. It receives 250 to 300 mm of rainfall per annum. Farming (Irrigated or/and floodwater) is the main economic activity of populations (Mangas and Mobeurs essentially). It is also a retreat zone for breeders during the dry season.
- Lake Chad zone: It is located in the far Eastern part of the Region in Lake Chad Basin. It receives 250 to 300 mm of rainfall per year. Floodwater farming is the main economic activity of communities (Mangas and Budumas) in the zone. This is a retreat zone by excellences for breeders.

#### Sampling and conduct of the survey

Sampling was conducted with the household as survey unit (all individuals living under the same roof and sharing the same amenities of life). The sample includes 150 households with sedentary livestock (50 households per agro-ecological zone) and 150 households with mobile livestock (50 households per agro-ecological zone). Sedentary households were drawn at random on the basis of attendance lists established during focus groups conducted in different villages selected. Mobile households were randomly made encounters of the encampments in territories of the villages selected because of their mobility.

The survey was conducted from February 10th to April 5th 2012. But the difficulties of meeting mobile breeders in Komadugu zone at the time of our passage have changed the structure of the sample so that the results of counting give 147 sedentary households (55 in pastoral bowls zone; 50 in Komadugu zone and 42 in Lake Chad zone) and 152 mobile households (45 in pastoral bowls zone, 31 in Komadugu zone and 76 in Lake Chad zone). However, this modification does not affect the quality and focus of the study.

### Results and discussion

#### Composition of the livestock surveyed

Figure 4 shows the compositions of the livestock surveyed with a total number of 15,618 head of animals in majority dominated by small ruminants (57.6% of livestock surveyed).

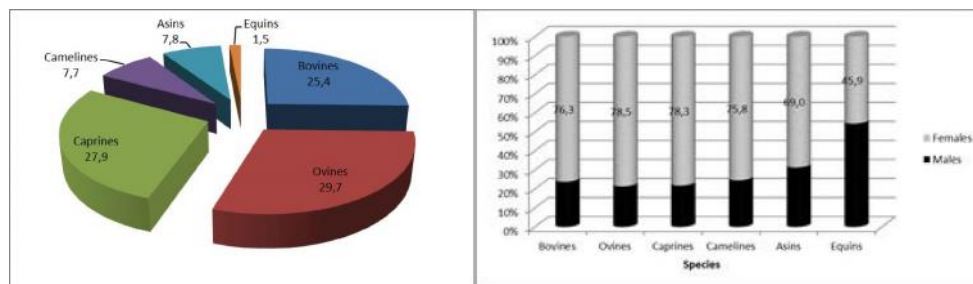


Figure 4. Composition of livestock population surveyed per species (%) Figure 5. Distribution of the livestock species per gender (%)

Livestock distribution per gender and species (figure 5) shows a preponderance of females (¾ of the herd) over males, all species combined, except horses where males represent 54.6% of their effective. Such a composition of the herds translates the strategic orientation of households towards livestock for breeding.

#### Composition of sedentary and mobile herds

Figure 6a shows that the sedentary herds in the Region of Diffa remain to large extent dominated by small ruminants representing 71.1% of livestock with a preponderance of goats (40.6%) over sheep (30.5%). Bovines and camels represent respectively 19.8% and 1.6% of sedentary herds. In mobile herds, small ruminants represent 51.8% of livestock with however a preponderance of sheep (29.3%) over goats (21.5%). Large ruminants contribute around 39% to mobile livestock numbers in the Region (Figure 6b).

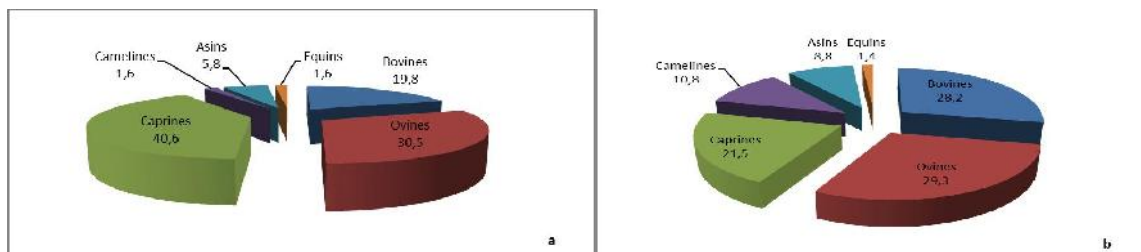


Figure 6. Comparison of the compositions of (a) sedentary herds and (b) mobile herds (%)

Composition herds according to agro ecological zones

Analysis of the composition of sedentary and mobile herds by agro ecological zone shows zonal disparities (figure 7). In the Lake Chad area, small ruminants represent 80% of sedentary herds against 72% in the pastoral bowls zone and 62% in the Komadugu zone (Figure 7a). These results indicate a trend towards capitalization in large animals such as cattle (29% of sedentary livestock), of Komadugu area sedentary households for whom animal husbandry is a secondary economic activity after agriculture. Households increasingly invest surplus generated by sales of agricultural products in cattle breeding. Such farming-livestock integration values rainfed and irrigated crop residues (rice straw) through cattle fattening.

Comparison of the compositions of mobile herds surveyed per zone (Figure 7b) shows a domination of small ruminants in Lake Chad (53%) and in the pastoral bowls zones (51%). In the Komadugu zone, large ruminants dominate (49.6%) with a high presence of camels unlike the two other areas certainly related to the period of survey.

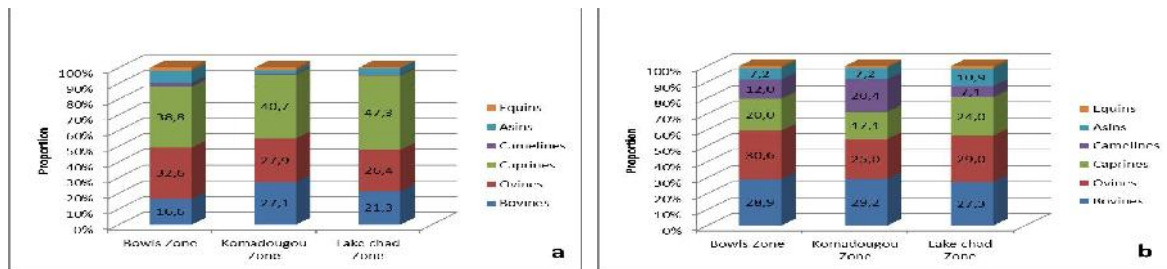


Figure 7. Comparison of (a) sedentary and (b) mobile herds by agro ecological zone (%)

Structure of livestock

Out of the 300 households surveyed, 270 (131 sedentary households and 139 mobile households) provided information considered exploitable for the purpose of this exercise. The total number of livestock population was 13,415 heads, including 3,625 cattle; 3,844 sheep; 3,883 goats; 786 camels; 1,096 donkeys and 181 horses.

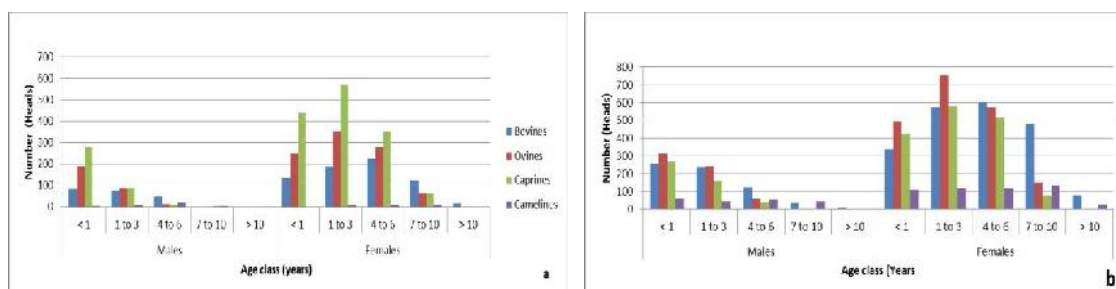


Figure 8. Structure of (a) sedentary and (b) mobile herds according to species and gender.

Analysis of the figure 8 shows the degree of systematic and early exploitation of male in comparison to female individuals, ceteris paribus. The gap is much more significant in the 1 to 3 years age group and beyond. For all livestock identified, 7.5% of male small ruminants are 1 to 3 years old against 29.3% for females. For cattle, proportions are respectively 8.7%

and 21%. However, the level of exploitation of male individuals varies according to the sedentary or mobile status of the livestock. In sedentary herds, 64-73% of male small ruminants are less than one year old against 39.7% for cattle and 13.3% for camels. In mobile herds, the proportions are 51-57%; 38.7% and 29.4% respectively.

Such a structure characterizes a rearing system oriented towards reproduction both mobile and sedentary herds. This reproduction is ensured by a core of female spawners more or less stable and largely dominated by young ones (figure 7). In small ruminants, more than one third (1/3) of females (37.8% of ewes and 38.1% of she-goats) are 1 to 3 years old. Thus, considering the age of first birth in large ruminants 45 to 48 months, we may say that reproduction in cattle and camels is ensured respectively at 54.1% and 42.2% by females aged 4 to 6 years. Females aged 7 to 10 years old represent respectively 39.6% and 47.5% and very few are above 10 years of age.

### **Discussion**

Such results translate a change in the composition and structure of the livestock population in the Region of Diffa which is characterized by a more or less significant dominance of small ruminants (especially goats). In 1970, according to the official human populations and livestock census, small ruminants represented 56.8% (sheep 21.3% and goats 35.5%) of herd in Diffa against 37.6% for cattle. Camels, asins and equines represented respectively 2.5%, 2.1% and 1% of herd in the Region (Republic of Niger, 1970). In 1978, small ruminants represented 62.4% of herd again 30.2% for cattle; 3.4% for camels; 2.8% for donkeys and 1.2% for horses (Republic of Niger, 1979). According to the results of the 2004-2008 Census in the Region of Diffa, small ruminants represented 65.3% of sedentary herds (ovine 24.9% and caprine 40.4%) and 39.6% of mobile herds (ovine 20.4% and caprine 19.3%).

The results of the livestock population structure resemble those obtained by Faugère and al. (1990a et b) in “Traditional small ruminants rearing system in Senegal...” marked the one hand, by scarcity or absence of older animals particularly the males, and secondly, by a domination of females regardless of the age group considered. The structure of cattle herds in sedentary areas of Diffa at the end of 1982 shows that 64.1% of females are aged 4 years and above; 23.9% are 1 to 3 years old and 12% are 0 à 1 year old. In the pastoral zone, the proportions are respectively 59.4%; 26.7% and 13.8%. 61.2%; 27.1% and 11.7% (PENCE, 1985).

In a nutshell, it is very rare to find in the Region of Diffa small ruminants of more than 7 years of age or cattle of more than 10 years of age. This is attributable to a large extent to the series of recurrent fodder and food deficits the Region has experienced for more than a decade (cf. figure 2). To this is added the occurrence of epizootic diseases (pleuropneumonia, piroplasmosis, etc.) which further weaken already malnourished livestock.

### **Conclusion**

Pastoralism in the Region of Diffa is changing in terms of livestock practices in the face of recurrent and even structural fodder and rainfall deficits. Over the years, many breeders have witnessed a change in the composition and structure of their herds. The share of cattle has

decreased in favor of small ruminants (especially goats) which are more resilient to climate shocks by feeding, where necessary, on aerial pastures. However, in the Komadugu zone, sedentary herds contain a much more significant proportion of cattle (29%) in comparison with other zones.

Livestock structure is characterized by a core of young spawners more or less stable. To exploit his flock, the breeder selects preferably male then female aged and / or sick. This strategy enters in the reproductive process and capital livestock accumulation in order to ensure the reproduction of flocks and milk production, and secondly, to keep potentially powerful and resistant animals in an environment marked by structural deficit rainfall and forage causing significant losses of animals and the pauperization of breeders.

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## INFLUENCE OF GENISTEIN AND ZEARALENONE ON BOAR SPERMATOZOA MOTILITY *IN VITRO*

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### Abstract

Genistein (GEN) belongs among soya phytoestrogens, while zearalenone (ZEA) is a metabolite of the fungi *Fusarium spp.* Both compounds can bind to estrogen receptors, so they can mimic the functions of endogenous estrogens. Pigs very often come into contact with these substances through feeding. The aim of this study was to evaluate the influence of GEN and ZEA on the motility of porcine spermatozoa by the CASA system (computer assisted sperm analysis). The effects of GEN and ZEA, concentration range 0,5 – 20  $\mu\text{M}$ , on the model of boar diluted semen were tested during 2- and 4-hour incubation at 38°C. The data obtained show that GEN and ZEA negatively affect sperm velocity parameters (VCL, VAP, VSL) in a dose and incubation period dependent manner. A significant decrease in sperm velocity parameters was recorded after addition of all the GEN or ZEA concentrations tested. A temporary increase in value was observed in the case of VSL only - after 2 hours of incubation with 0,5 – 10  $\mu\text{M}$  ZEA. Also, a dose dependent increase of immotile spermatozoa corresponded to changes of the sperm velocity. In conclusion, even relatively low doses of the substances tested can negatively affect pig fertility.

**Keywords:** Pig, sperm, genistein, zearalenone, CASA

### Introduction

The quality of boar semen is influenced by various endogenous and exogenous factors such as hormonal background or nutrition, and nutrition can already serve as a pathway for the admission of hormonal active compounds which can affect both male and female.

In this way spermatozoa can be exposed to environmental estrogens which are structurally similar to 17 $\beta$ -estradiol and show estrogenic activity. Among these substances soy phytoestrogens and zearalenone belong. The richest source of soy phytoestrogens are soybeans (*Glycine max*) (Moravcová et Kleinová, 2002). Feedstuff for pigs contains up to 18% soybeans (Zeman et al., 2006). Soy phytoestrogens taxonomically belong among isoflavones, the most important of which is genistein, which is, among other things, a known inhibitor of tyrosinprotein kinases (Bajpal et al., 2003). Zearalenone (ZEA) is a mycotoxin produced by *Fusarium* moulds (Minervini et Dell'Aquila, 2008). Livestock are exposed to its effects in feed, and pigs are considered the most sensitive species (Benzoni et al., 2008; Fink – Gremmels et Malekinejad, 2007; Agag, 2004; Ravishankar et Karim, 2010). The mechanism of action of both genistein and zearalenone involves their binding to estrogen receptors. In an organism they can demonstrate both estrogenic and antiestrogenic activity (Tapiero et al., 2001; Whitten et Patisaul, 2011). Whether estrogenic and antiestrogenic effects occur in the organism depend not only on the concentration of environmental estrogens, but also on the momentary level of endogenous estrogens (Tapiero et al., 2002) and the type of ER (Minervini et Dell'Aquila, 2008; Tapiero et al., 2002).

Assessment of mature spermatozoa *in vitro* provides an appropriate model for studying the influence of the environmental estrogens mentioned above, which can occur in

the female genital tract as a result of the consumption of soy grits or feed with zearalenone contamination. A comparison of genistein and zearalenone effects on boar spermatozoa has not been published yet.

CASA – computer assisted sperm analysis is a practical tool for objective analysis of sperm motility and for classification of various motion categories (Kathiravan et al., 2011). This system is based on capturing consecutive images from microscope by simple chip camera (Quintero – Moreno et al., 2003), and the image obtained is consequently exported to a computer. Image assessment is provided by specific software with the CASA module, which is able to analyse them (Allahbadia, 2005). The data obtained are mathematically processed and individual trajectories are defined in numerical form. The results are expressed by a set of parameters, which accurately define the motion of sperm cells (Quintero – Moreno et al., 2003).

The aim of this study was to assess the motion parameters of boar spermatozoa *in vitro* by CASA.

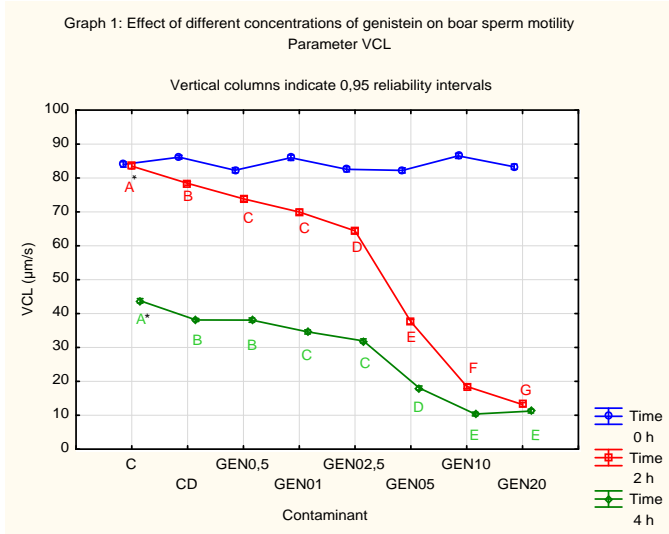
### **Materials and methods**

Spermatozoa were obtained from France hybrids boars as commercial short – term preserved insemination doses. The doses were kept during transportation and storage at 17°C and were processed on the day of collection or on the following day. Genistein (GEN) and zearalenone (ZEA) were dissolved in DMSO (dimethyl sulfoxide) to a final concentration of 2µl DMSO/ 998 µl ejaculate and 0,5; 1; 2,5; 5; 10; 20 µM GEN or ZEA. The acquired results were confronted with pure control and control with DMSO. All of the instruments and laboratory equipment were heated to 38°C. For control, all samples were examined after 4 min incubation before the addition of GEN or ZEA. Spermatozoa were incubated for 2 and 4 hours at 38°C in a waterbath. The samples were then injected into the chamber of calibrated Leja® microscopic slide and assessed by a Nikon E 600 microscope with a heating plate, objective lens PH 10x with negative phase contrast and a Jenoptik Prog Res CT1 camera. Capturing and image analyses were performed using the software Nis Elements 3.2 (Laboratory Imaging, Prague). The parameters assessed were VCL (curvilinear velocity), VSL (straight line velocity) and VAP (average path velocity). Based on the parameters obtained, spermatozoa were divided into the categories of motile and immotile. Distinct differences at the level of velocity parameters can be related to the high sensitivity of the CASA system. Choosing a significance level of  $p= 1 \times 10^{-6}$  should be sufficient for the separation of residual variability and the experimental scheme of variability. For the statistical analysis program Statistica.cz version 10 was used. All data were subjected to multifactorial ANOVA system.

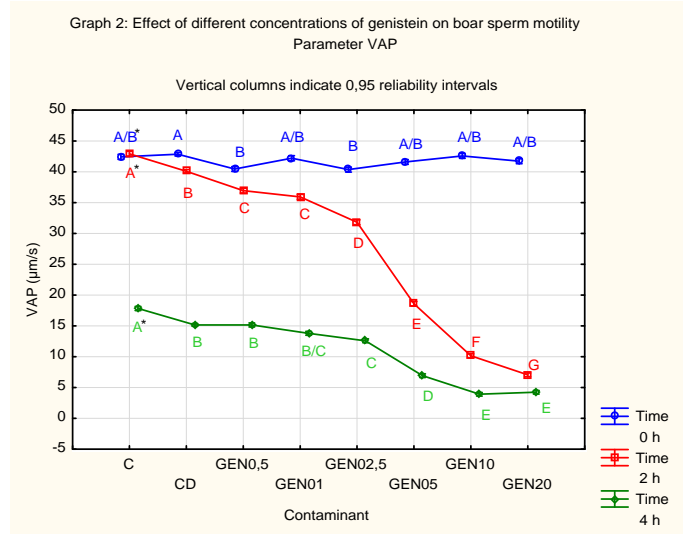
### **Results and discussion**

Our data show that both of the tested contaminants considerably affected the values of motion parameters of boar spermatozoa. In our experiment a statistically significant decrease of sperm motility was observed after addition of various concentrations of GEN already 2 hours after incubation. This was confirmed by a rapid decrease of motion parameters marked mainly from the concentration of 5 µM (graphs 1 – 3). A significant increase in immotile spermatozoa, especially in the samples with 10 and 20 µM supplementation (graph 4) correlates with this fact. After 4 hours incubation another decrease in velocity motion parameters in a dose dependent manner was recorded in all samples (graphs 1 – 3). Related increase in the immotile sperm ratio was also observed (graph 4).

Graph 1



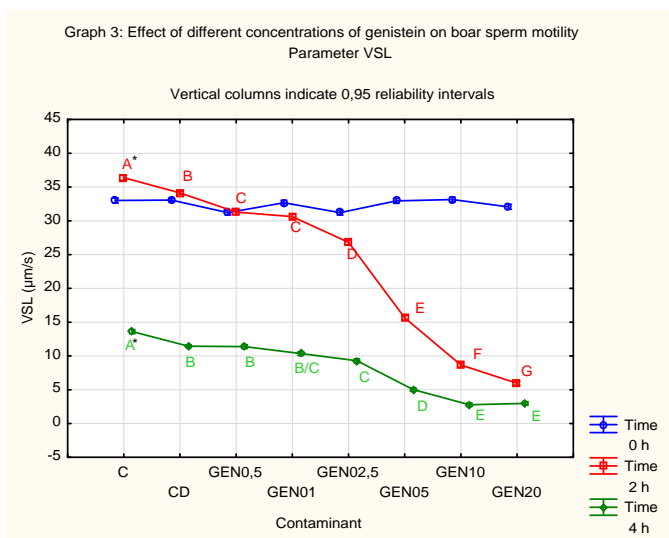
Graph 2



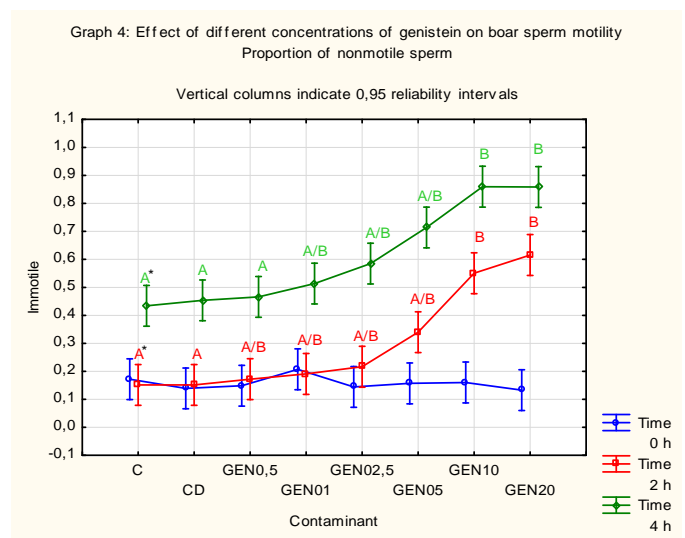
\*values marked with different indices of the same color are statistically significantly different at a significance level  $p < 1 \times 10^{-6}$

\*values marked with different indices of the same color are statistically significantly different at a significance level  $p < 1 \times 10^{-6}$

Graph 3



Graph 4



\*values marked with different indices of the same color are statistically significantly different at a significance level  $p < 1 \times 10^{-6}$

\*values marked with different indices of the same color are statistically significantly different at a significance level  $p < 0,05$

Several studies have been concerned with the influence of genistein on sperm motility. However, they were conducted on different animal models with various experimental protocols, so the results obtained are ambiguous. While Adeoya – Osiguwa et al. (2003), Eustache et al. (2009) and Martinez – Soto et al. (2010) demonstrated that genistein has an influence on sperm motility, Pukazhenti et al. (1998), Hinsch et al. (2000) and Menzel et al. (2007) obtained different results. None of these author collectives worked with boar sperm. Our work is in methodology most relevant to the study of Martinez – Soto et al. (2010), which described the influence of freezing and thawing media with the addition of GEN on human sperm. The tested concentrations were 0,1 µM, 1 µM and 10µM GEN in the freezing medium



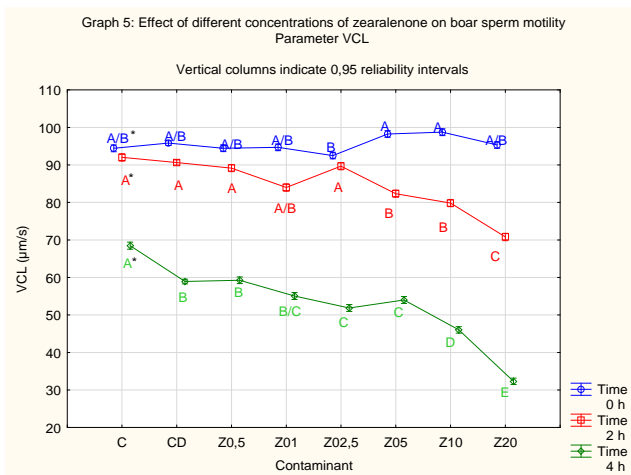
and 1  $\mu\text{M}$  and 10  $\mu\text{M}$  in the thawing medium. Sperm were thawed at a constant temperature of 37°C, incubation received 60 min. In agreement to our results Martinez – Soto et al. (2010) recorded a decrease in motile spermatozoa and progressive motile spermatozoa in a dose dependent manner both in the freezing and thawing medium. In the case of the thawing medium decrease of VCL, VSL and VAP was also observed. Also, publications by a Hinsch et al. (2000) and Menzel et al. (2000) were similar to our experiment concerning the temperature and concentration of genistein. They worked with cryopreserved bovine spermatozoa. Hinsch et al. (2000) worked with concentrations of GEN of 0,74  $\mu\text{M}$  and 7,4  $\mu\text{M}$ , and samples after thawing were kept at 38°C. Menzel et al. (2007) worked with concentrations of GEN of 0,074  $\mu\text{M}$ , 0,74  $\mu\text{M}$  and 7,4  $\mu\text{M}$ , and samples after thawing were kept at 38,5°C. In both studies GEN did not affect sperm motility. However, bovine spermatozoa can be less sensitive to this phytoestrogen than porcine spermatozoa.

In our experiment zearalenone as well as genistein inhibited sperm motility in a concentration dependent manner.

After 2 hours of incubation ZEA induced a decrease in the velocity motion parameters statistically significant at 5 – 20  $\mu\text{M}$  concentrations. However, in the case of VSL except 20  $\mu\text{M}$  ZEA an increase in this parameter was recorded. Differences between pure control, control with DMSO and samples with 0,5 – 2,5  $\mu\text{M}$  of ZEA were minimal. Significant deviations were observed after the addition of 5 – 20  $\mu\text{M}$  ZEA (graphs 5 – 7). The ratio of immotile spermatozoa continuously increased in all tested groups with the highest manifestation in the sample with 20  $\mu\text{M}$  ZEA (graph 8),

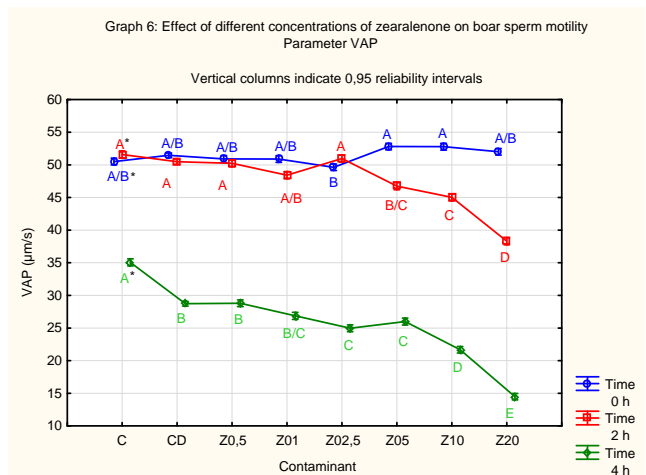
After 4 hours of incubation a rapid velocity decrease at all the concentrations tested occurred in a dose dependent manner. Simultaneously the differences between control and experimental samples were more marked, mainly at 10 and 20  $\mu\text{M}$  ZEA (graphs 5 – 7). An increase in immotile spermatozoa in a dose dependent manner was also determined, however, this effect was not statistically significant (graph 8).

Graph 5



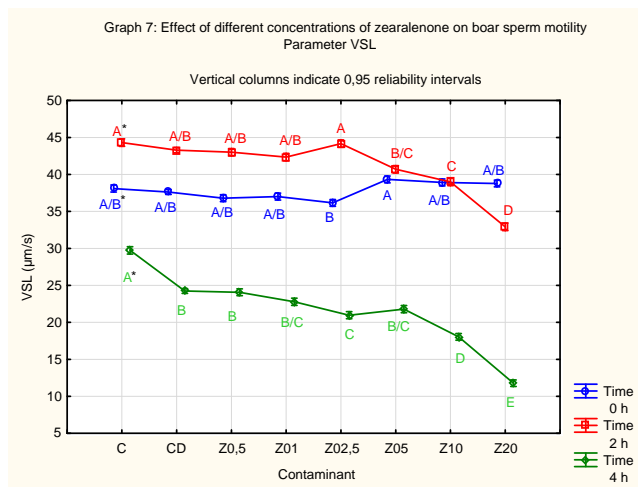
\*values marked with different indices of the same color are statistically significantly different at a significance level  $p < 1 \times 10^{-6}$

Graph 6



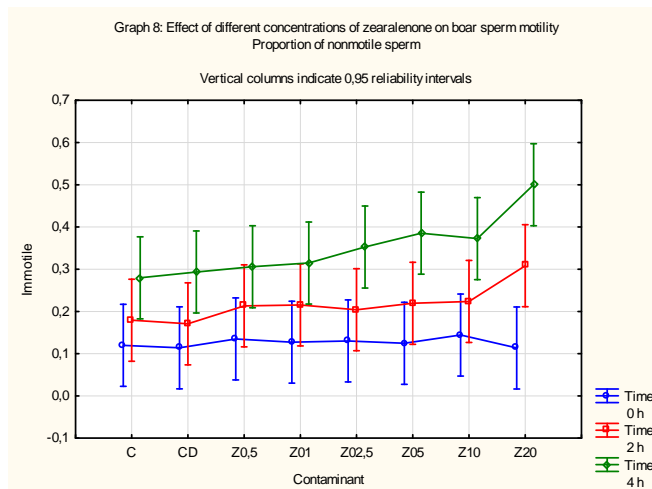
\*values marked with different indices of the same color are statistically significantly different at a significance level  $p < 1 \times 10^{-6}$

Graph 7



\*values marked with different indices of the same color are statistically significantly different at a significance level  $p < 1 \times 10^{-6}$

Graph 8



\*values marked with different indices of the same color are statistically significantly different at a significance level  $p < 0,05$

ZEA and its influence on the motility of boar spermatozoa has been the focus of attention of several authors. This substance was tested in a wide spectrum of concentrations from picomolar to milimolar amounts. However, most of studies used higher concentrations than in our experiment. The negative effects of ZEA on sperm motility parameters were confirmed by Rajkovic et al. (2007) who incubated boar sperm with a very high amount of ZEA 3,1 – 310 mM, although concentration 3,1 was not effective. The effects on motility were expressed as a decrease of percentage of progressive motile spermatozoa. Tsakmakidis et al. (2006, 2007) assessed the effect of ZEA after the addition of 125 µM, 187,5 µM and 250 resp. 40 µg/l, 60 µg/l and 80 µg/l. The incubation periods were 1, eventually 2, 3 and 4 hours at 38,5°C. A significant decrease in motility was observed after 1 hour of incubation (Tsakmakidis et al. 2006, 2007), and capturing after each following hour led to another depression in sperm progressive motion. After 4 hours of incubation steep decline in motility was observed. In the case of 250 µM, the percentage of progressive motile spermatozoa was almost zero (Tsakmakidis et al., 2006). This effect was dose dependent, which corresponds to our results.

However, in the study of Tsakmakidis et al. (2008) no effect on sperm motility after the addition of 31,4 µM, 62,8 µM or 94,2 µM ZEA was observed. Incubation conditions were similar to previous studies at 38,5°C/ 4 hours. This contradiction of our results could be caused by the different method of sperm motility assessment. While Tsakmakidis et al. (2008) assessed motility by subjective evaluation, we used an objective system of computer assisted sperm analysis (CASA).

Lower concentrations of ZEA (0,2 pM – 20 µM) were tested by Benzoni et al. (2008). In this study the CASA system for motility evaluation was used. Contrary to our results, no effects on VCL and VSL after 5, 16 and 24 h incubation were observed. This contrast could be caused by the different temperature during incubation. Benzoni et al. (2008) incubated spermatozoa at 18°C, which corresponds to the preservation temperature, while our samples

were maintained at 38°C, which corresponds to the body temperature recommended by Verstegen et al. (2002).

### Conclusion

In our experimental conditions genistein significantly inhibited the motility of boar spermatozoa in a dose dependent manner from 0,5 µM, and zearalenone had the same negative effect from concentration 5 µM. Contrary to published studies, in our experiments *in vitro* the effects of both substances were confirmed at a relatively low concentration. As GEN is a considerable part of pig nutrition and ZEA mycotoxicosis is a common problem at pig farms, it is possible that these compounds can affect reproduction also *in vivo*.

### Acknowledgment

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## INFLUENCE OF DAIDZEIN ON MEIOTIC MATURATION OF PIG OOCYTES

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### Abstract

Daidzein is ranked among phytoestrogens, natural substances which influence an organism due to their binding to estrogen receptors, similarly to endocrine disruptors. For that reason, their effects are manifested especially in the field of reproduction. One of the important presumptions of female reproduction is a physiological course of meiotic maturation of oocytes. The high portion of soya in a pig's diet predetermines this species for the possible influence of daidzein on reproductive functions. The aim of this study was to determine the effects of an increasing concentrations of daidzein on the meiotic maturation progress of porcine oocytes under *in vitro* conditions. The oocytes were exposed to different concentrations of daidzein (10, 20, 40, 50µg/ml). After 24 hours cultivation the stage of nuclear maturation and the area of cumulus – oocyte complexes reached as a detector of cumular cell expansion were evaluated. The high level of daidzein's solubility was determined by the HPLC method.

The effects of daidzein on oocytes were already manifested at the lowest concentration used. Nuclear maturation was inhibited in a dose dependent manner. The maximum effects were observed in the concentration of 20µg/ml, and inhibitory effects of higher concentrations were determinate at the same intensity. Daidzein suppressed the expansion of cumular cells as well. The lowest (10µg/ml) and highest (50µg/ml) concentrations had the strongest effects on cumular expansion. In conclusion, daidzein negatively influences the meiotic maturation of porcine oocytes, whereas the expansion of cumular cells reacts more sensitively than nuclear maturation of oocytes to this soya phytoestrogen.

**Keywords:** phytoestrogen, daidzein, pig, meiosis, oocyte

### Introduction

Phytoestrogens are related to a number of other natural, but also synthetic, substances, classified among so - called endocrinous disruptors – substances with a similar chemical structure to estradiol. This enables them to bind to estrogen receptors and elicit estrogenic or anti - estrogenic activities (Holoubek and adová, 2000; Tham et al., 1998). Estrogenic activity of phytoestrogens is dramatically lower than 17 β- estradiol. However, their concentration in an organism may be markedly higher compared to endogenous estrogens (Tham et al. 1998). Therefore, the effects of phytoestrogens can be manifested in an organism very intensively.

Many livestock are exposed to the influence of phytoestrogens, because they especially appear in clover and legumes. This was confirmed in grazing ruminants, but also in pigs fed with a soya diet. The effects of phytoestrogens on animals are mainly unfavorable, in contrast to the human organism, and they especially affect the area of reproduction (Adams 1995). In many livestock, the effects of phytoestrogens on sexual differentiation and gonad development, as well as on abortions, were documented (Romero et al., 2008). Pathological changes in the reproductive system (Adams, 1996; Burton and Wells, 2002), disturbances of

the estrous cycle (Adams 1995), infertility and a number of reproductive problems, including negative effects on gametes, were also observed (Burton and Wells, 2002; Kala and Mika 1997; Kurzer and Xu, 1997; Rosselli et al. 2000; Dusza and Ciereszko, 2006).

The meiotic maturation of oocytes is one of the important moments of reproduction. It can be influenced by many internal and external factors, and its physiological course is fundamental for successful fertilization and resulting embryonic development. Experimentally, under *in vitro* conditions, it was found, that the addition of the naturally occurring endogenous hormone 17  $\beta$ - estradiol inhibits the meiotic maturation of porcine (Li et al., 2004) and bovine oocytes (Beker et al., 2002). Various phytoestrogens affect oocytes similarly, but the intensity of their effects and their negative impact on the course of meiotic maturation are different.

Meiotic maturation was inhibited after the addition of another phytoestrogen – genistein – to the cultivation medium. This was confirmed in several animal species (Jung et al., 1993; Makarevich et al., 1997; Vodková et al., 2008 – pig, Van Cauwenberge and Alexander, 2000; Yoshida et Mizuno, 2012 – mouse). On the other hand, when low doses of genistein were added, the meiotic maturation of porcine oocytes was stimulated (Makarevich et al., 1997). Daidzein, similarly to genistein, has an inhibitory effect on the meiotic maturation of mouse oocytes. Nevertheless, the inhibitory effect of daidzein is markedly lower in comparison with genistein (Van Cauwenberge and Alexander, 2000). From the papers by Van Cauwenberge and Alexander (2000) and Yoshida et Mizuno (2012) it seems that it is difficult to prove the daidzein effects on mouse oocyte nuclear maturation at doses lower than 50  $\mu$ M. The daidzein effects on pig oocyte maturation were not found (Galeati et al., 2010) but very low doses were tested in this experiment (1 a 10 $\mu$ M).

In general, the cumular cells also play an important role in the effect of estrogenic substances on oocytes, including their meiotic maturation. These cells enclose an oocyte in the antral follicle and harbor an estrogen receptor, similarly to oocytes (Wassarman and Albertini, 1994). The importance of cumular cells consists in the regulation of interruption and resumption of meiosis during oocyte development and in the support of the subsequent cytoplasmatic maturation of the oocyte (Tanghe, 2002). During meiotic maturation the cumular cells synthesize the structural components of the extracellular matrix, whereby their expansion and increase of the total cumulus-oocyte complex (COC) takes place (Eppig, 1979).

Similarly to nuclear maturation, expansion is influenced by estradiol (Li et al., 2004) as well as by some phytoestrogens. Whereas genistein inhibits the cumular cell expansion of oocytes in mice (Tirone et al., 1997) and in pigs (Jung et al., 1993; Vodková et al., 2008), in daidzein such data are missing. Nevertheless, the decrease of progesterone production during pig oocyte *in vitro* maturation noted by Galeati et al. (2010) indicates the effects on cumular cells activity, even at above mentioned very low daidzein concentrations.

The aim of this experiment was to determine the influence of various concentrations of daidzein on meiotic maturation of pig oocytes after 24 hour *in vitro* cultivation. The achieved stage of nuclear maturation reached and the area of expanded cumular cells or the entire cumulo-oocyte complex (COC) were evaluated.

## Materials and Methods

### Acquisition of ovaries

Porcine ovaries were obtained from non-cycling gilts at an unknown phase of estrus cycle at a slaughterhouse. The ovaries were transported to the laboratory in a saline solution (0.9 % NaCl) at 39 °C. Oocytes were collected from the ovarian follicles (3 – 5 mm) with a 20-gauge aspirating needle. Only fully-grown oocytes with intact cytoplasm and compact cumuli were used in the experiments.

#### Selection, cultivation and evaluation of oocytes

The oocytes were cultivated in modified M199 medium (Sigma-Aldrich, USA), containing sodium bicarbonate (32.5 mM), calcium L-lactate (2.75 mM), gentamicin (0.025 mg/ml), HEPES (6.3 mM), 13.5 IU eCG: 6.6 IU hCG/ml (P.G.600; Intervet, Holland) and fetal calf serum (8,14g/l) in the amount of 20µl/ml cultivation medium (BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, a.s.). The oocytes were matured in 4 – well dishes (Nunc, Roskilde, Denmark) containing 1 ml of culture medium at 39 °C in an air mixture of 5.0 % CO<sub>2</sub>. The oocytes were cultivated for 24hours to metaphase I (MI) with the addition of relevant dose of daidzein (Sigma Aldrich) in concentrations 0, 10, 20, 40, 50µg/ml. After cultivation a digital image of the oocytes and their cumular cells (COC) was recorded. Then the cumular cells were removed by pipetting through a narrow glass pipette. The oocytes were fixed in a solution of acetic acid and ethanol (1:3).

The area of COC was measured and evaluated by digital image analysis by NIS-Elements software (AR, 3.10, Laboratory Imaging, CZ). Nuclear maturation was evaluated under light microscope after staining the oocytes with 1% orcein.

#### Statistical analysis

The oocytes were subjected to further evaluation only if at least 85% of the control group oocytes reached the MI stage after 24hours cultivation. For statistical evaluation of COCs the Kruskal – Wallis ANOVA test was used, and for nuclear maturation, the chi-square test.

#### Analysis of solubility by HPLC method

The actual concentrations of daidzein in the solutions created were determined using the HPLC technique with UV detection. The HPLC SUMMIT instrumentation (automated sample injector ASI-100; thermostatted column compartment TCC-100; dual gradient pump P680 and photodiode array detector PDA-100) was manufactured by Dionex (Germany and USA) and operated by software Chromeleon (Dionex, Germany). A Gemini C18 column, 5 µm, 110A, 4.6×250 mm with a guard column (Phenomenex, Torrance, CA, USA) was used, and the column temperature was set to 45 °C. Binary gradient elution was used using 100% acetonitrile (ACN) (LAB-SCAN - Gliwice, Poland) and 0.1% trifluoroacetic acid (TFA) (Sigma-Aldrich) as the mobile phase at a ratio of 15 : 85 rising to 70 : 30 over 40 min; the flow rate was 0.8 mL min<sup>-1</sup>. The UV detection was performed at a wavelength of 260 nm (Leuner et al.2013).

### Results and discussion

The pig oocytes were exposed to daidzein for 24 hours, and its influence on meiotic maturation, nuclear maturation and expansion of cumular cells was evaluated *in vitro* (Table 1, Table 2.)

#### 1. Influence of daidzein on nuclear maturation of oocytes

Daidzein blocked nuclear maturation in all concentrations tested. The lowest concentration of daidzein (10µg/ml) in the cultivation medium significantly ( $P < 0.05$ ) affected the nuclear maturation of oocytes, when expressive retardation occurred and oocytes after 24 hour cultivation did not reach the MI stage, but only late diakinesis (LD) or premetaphase (PM). When higher concentrations of daidzein (20, 40, 50µg/ml) were used, significantly more oocytes ( $P < 0.05$ ) did not begin nuclear maturation and remained in the germinal vesicle (GV) stage, where there was no difference in intensity of effect between individual concentrations (Table 1).

Table 1 – Nuclear maturation of oocytes after 24hours cultivation with daidzein

Meiotic maturation stage (%)	Concentration of daidzein				
	0µg/ml	10µg/ml	20µg/ml	40µg/ml	50µg/ml
GV	3 <sup>A</sup>	4 <sup>A</sup>	14 <sup>B</sup>	12 <sup>B</sup>	14 <sup>B</sup>
LD+PM	1 <sup>A</sup>	10 <sup>B</sup>	4 <sup>AB</sup>	8 <sup>B</sup>	11 <sup>B</sup>
MI	88 <sup>A</sup>	85 <sup>A</sup>	72 <sup>B</sup>	74 <sup>B</sup>	71 <sup>B</sup>
MII	5 <sup>A</sup>	0 <sup>B</sup>	5 <sup>A</sup>	2 <sup>A</sup>	2 <sup>A</sup>
Ab+deg	1 <sup>A</sup>	1 <sup>A</sup>	5 <sup>A</sup>	4 <sup>A</sup>	2 <sup>A</sup>
Number of oocytes	159	97	125	100	104

GV – germinal vesicle, LD + PM – late diakinesis + premetaphase, MI – metaphase I, MII – metaphase II, Ab. + deg. – abnormal and degenerate oocytes.

<sup>A,B</sup> – values with different superscripts in the row differ on the level ( $P \leq 0,05$ )

## 2. Influence of daidzein on expansion of cumular cells

The degree of cumular cell expansion is expressed as the mean area of cumulo-oocyte complexes (COC) in percentage compared to the area of COC cultivated in a non-supplemented medium (Table 2).

In oocytes cultivated during meiotic maturation together with daidzein, the COC was smaller in all concentrations tested ( $P < 0,05$ ). The most effective concentrations were the lowest and highest (10 and 50µg/ml), as the oocytes reached 52% and 37% of the area of COC when compared to the control group.

Table 2 – Area of cumulus oocyte complexes (COC) after 24hours cultivation with daidzein

Concentration of daidzein (µg/ml)	Average of areas COC (%)
0	100 <sup>A</sup>
10	52 <sup>B</sup>
20	70 <sup>C</sup>
40	64 <sup>C</sup>
50	37 <sup>D</sup>

<sup>A,B,C,D</sup> – values with different superscripts in the row differ on the level ( $P \leq 0,05$ )

In this study we demonstrated a negative influence of daidzein on meiotic maturation of pig oocytes under *in vitro* conditions. Nuclear maturation of oocytes was inhibited from the lowest concentration (10µg/ml) used. This concentration inhibited nuclear maturation partially, because only 10% of the oocytes achieved late diakinesis, not the MI stage. A higher concentration of daidzein (20µg/ml, if you like 80µM) already prevented the germinal vesicle breakdown in the course of meiotic maturation.



Van Cauwenberge and Alexander (2000) used concentrations similar to our experiment (Dai 50, 100, 200 $\mu$ M) and also noted inhibitory effects of daidzein on nuclear maturation of mouse oocytes, as well as Yoshida and Mizuno (2012) did at a dose of 100 $\mu$ M of daidzein. However, in the experiments mentioned the authors found a markedly higher percent of inhibited oocytes. This can indicate that porcine oocytes might be less sensitive to daidzein's effects than mouse oocytes are. Contemporarily it seems, the lack of daidzein effect on pig oocyte nuclear maturation in the paper by Galeati et al. (2010) was really related to low concentration used not to the properties of daidzein itself.

We also demonstrated the effect of daidzein on cumular cell expansion from the lowest concentrations used. The inhibitory effect was not dose dependent and maximum intensity was reached when using the lowest and highest concentration of the supplement.

The results of studies concerned with the influence of daidzein on cumular cell expansion are lacking. Nevertheless, the inhibition of this phenomenon observed in our study agrees with the decrease of progesterone production during pig oocyte *in vitro* maturation published by Galeati et al. (2010). Also, the results can be compared with another flavonoid with an affinity to estrogen receptors, which is genistein. This substance also significantly suppresses expansion of the cumulus (Vodková et al., 2008). In contrast to daidzein, the inhibitory activity of genistein was observed in a dose dependent manner. The effect of genistin, a glycoside form of genistein, on cumulus expansion was markedly weaker (Vodková et al., 2008) and similarly to daidzein did not suppress cumular cell expansion in a dose dependent manner (Vodková et al., 2008). Neither daidzein nor genistin in contrast to genistein is considered a tyrosine protein kinase inhibitor (Jung et al., 1993). It is possible that this very fact can play a role in the general trend of incidence of these substances. The absence of dose-dependence of daidzein and absence of an effect on cumular expansion can be due to the phenomenon referred to as low-dose effect, which is typical for many endocrine disruptors (Vandenberg et al., 2012).

### **Conclusion**

Daidzein acted in an inhibitory manner on the indicators we monitored in our study – nuclear maturation and expansion of cumular cells. The inhibitory effects of daidzein were not dose dependent, and the effects of a series of increasing concentrations were manifested more intensively on the expansion of cumular cells than on the nuclear maturation of oocytes.

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## INFLUENCE OF LDL ADDITION ON CRYOPROTECTIVE PROPERTIES OF BOVINE SEMEN EXTENDERS

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### Abstract

The cryopreservation process and subsequent thawing of insemination doses (ID) can impair spermatozoon structures, especially sperm acrosome, nucleus and plasma membrane. These alterations affect post-thaw sperm motility, which is considered as the most important indicator in terms of fertilization capability (quality) of ID. The above-mentioned changes can be minimized through the usage of an appropriate cryoprotective compound in semen extender. Generally effective egg yolk has a number of disadvantages. Nevertheless, low density lipoprotein (LDL) - responsible for its cryoprotective effects - could improve the properties of the currently used non-yolk extenders.

The objective of this work was to evaluate the effect of the combination of LDL, processed from egg yolk plasma, with the commercially produced extender Andromed® (Minitübe, Germany) containing plant phospholipids. LDL was added to the extender at concentrations of 4%, 6 %, 8 %. Sperm motility was assessed using the selected Computer Assisted Sperm Analysis parameters (VCL, VAP, VSL) after 2 hours of incubation at 37 °C. After addition of LDL, the evaluated parameters reached significantly higher values compared with the control samples before cryopreservation and post-thawing as well. However, no differences were found among added LDL concentrations.

It can be concluded that the addition of LDL to an extender with plant phospholipids can have positive effects on sperm motility.

**Keywords:** LDL, spermatozoa, motility

### Introduction

During the process of freezing and thawing of insemination doses (ID) the viability and fertility of sperm cells are reduced mainly due to the negative effects on sperm structure and physiological processes within the cells (Amirat et al., 2004).

The causes of these changes are represented by a combination of influences, such as congenital low resistance to the effects of cryopreservation, the technique of dilution and the semen freezing protocols (Holt, 2000; Medeiros et al., 2002). The structure of the sperm which is primarily affected during cryopreservation is the plasma membrane (Hammerstedt et al., 1990). Structural changes in the plasma membrane during freeze-thawing play an important role in the reduction of fertilizing capacity during long-term storage of spermatozoa (Parks and Graham, 1992). The main goal of freezing protocols is to prevent these harmful effects, which can be achieved by, among other things, the usage of appropriate cryoprotective agents (Amirat et al., 2004).

In common practice different types of semen extenders are used, these can be divided according to the type of cryoprotective substance into skimmed-milk extenders, extenders on

a base of plant phospholipids, with the last group including extenders containing egg yolk (Vishwanath and Shannon, 2000). On the basis of the study by Pace and Graham (1974) it has been found that the component of egg yolk which is responsible for its cryoprotective properties is Low Density Lipoprotein (LDL). LDL consists of spherical particles mainly present in egg yolk plasma (85%), composed of a core made up of triglycerides and cholesterol esters surrounded by an outer layer consisting of phospholipids and apoproteins (Anton et al., 2003). The mechanism of the protective action of LDL has not yet been completely elucidated. However, the positive effects of LDL reported by Bergeron and Manjunath (2006) consist in the formation of strong and stable bonds with the major protein fraction of the bovine seminal plasma BSP protein family (BSP-A1/A2, BSP-A3 and BSP-30-kDa). These proteins trigger choline phospholipids and cholesterol efflux from the plasma membrane, thereby causing a decrease in the integrity and consequently an increase in sperm susceptibility to cold shock. Further beneficial effect of this substance is a positive correlation with the anti-oxidative system activity of sperm cells as demonstrated by authors Hu et al. (2011). Moreover the replacement of egg yolk only by its active fraction LDL can prevent microbiological risks, because, the results of the study Bousseau et al. (1998) showed that in spite of the antibiotic content in the composition of commercially produced egg yolk extenders, there was significant bacterial contamination. Simultaneously this would also eliminate the negative effects of other components in egg yolk (Pace and Graham, 1974).

The possibility of egg yolk replacement by the LDL fraction in commercially produced extenders and their effects on the motility of sperm cells during the freezing-thawing process has already been verified by several studies (Moussa et al., 2002; Amirat et al., 2004; Amirat et al., 2005; Amirat-Briand et al., 2010; Hu et al., 2010; Hu et al., 2011) with positive impact of 8% LDL concentration in all cases. Further, egg yolk extenders with the yolk replaced by the fraction LDL were compared with extenders on the basis of plant phospholipids (the both - IMV, L'Aigle, France): Bioxcell (Vera-Munoz et al., 2009) and Biociphos (Moussa et al., 2002; Amirat et al., 2005). However these studies did not show a significant difference.

The objective of this study was to assess the effect of the addition of LDL to the commercially manufactured plant phospholipid-based extender AndroMed® (Minitübe, Germany) on selected motility parameters of spermatozoa in native ejaculate and during the freezing – thawing process.

## Materials and methods

### *LDL extraction*

Low Density Lipoprotein (LDL) was prepared in accordance with the methodology of Moussa et al. (2002). Hen eggs were obtained from Biopharm Inc., and production of the fraction of LDL was ensured by the company Henna Inc.

Firstly, egg yolk had to be separate from albumen, manually, by rolling on filter paper in order to remove the endosperm and chalazae. Then the vitellin membrane was cut with a scalpel, and the egg yolks were collected in a beaker kept refrigerated by ice at 4 °C. In this way egg yolk plasma obtained was diluted by 0.17 M NaCl solution and then mixed at 4°C/1h. Afterwards centrifugation 10,000 x g for 45 min at 4 °C was performed and after supernatant removal, the procedure was repeated. The main purpose of this centrifugation was to achieve the separation of egg yolk granules from plasma. The obtained egg yolk plasma was stored at 4 °C. For removal of livetines 20.5 g of ammonium sulphate was added to 100 ml of plasma, and this was stirred for 1 hour at pH 8.7 and temperature 4 °C. Precipitated livetines were subsequently separated by centrifugation at 10,000 x g/45 min. Supernatant rich in LDL was dialyzed further 10 h for the purpose of ammonium sulphate elimination and selective coagulation of LDL. At the end of this dialysis the mixture was centrifugated at

10,000 x g/45 min, and the resulting sediment (pellets) represented LDL with a purity of 97%, which had to be stored at 4 °C.

#### *Preparation of the extenders*

Two extenders were prepared just before the beginning of the experiments. The commercially produced non-egg yolk extender AndroMed® (Minitübe, Germany) was used as the control, composition: plant phospholipids, Tris, citric acid, sugars, antioxidants, buffers, Tylosin, Gentamicyn, Spectinomycin, Lincomycin (the quantity of antibiotics in accordance with regulation EC 88/407). It was prepared by the standard method according to the instructions of the producer, deionized water preheated to 30-32 °C was added to the concentrated solution of the extender at the ratio 4 : 1. The extender enriched by LDL was prepared in the same manner only with the difference of 4%, 6%, or 8% LDL addition.

#### *Collection and processing of semen*

The semen was collected randomly from 7 bulls at the insemination centre in the standard way. It was submitted to the assessment of ejaculate volume, sperm concentration ( $\geq 0.7 \times 10^9$ /ml), and percentage of motile sperm ( $\geq 70$  %). All samples conformed to the limits. Afterwards the ejaculate was transported in a cooling box at a temperature of 6-8 °C to the laboratory for further processing.

The ejaculate was diluted to a concentration of approximately  $30 \times 10^6$ /ml, which has been proven for optimal evaluation of motility using Computer Assisted Sperm Analysis (CASA) (Verstegen et al., 2002). Cryopreservation was carried out in the laboratory; straws were filled by a pipette in the styrofoam box at 6-8 °C and sealed by preheated pean. After filling, the straws were stored in a refrigerator for a time of equilibration - 4 hours. Then the straws were inserted into the styrofoam box, adjusted for cryopreservation, frozen in nitrogen vapor (4 cm above the surface) for 10 min at a temperature of approximately -120 °C and subsequently immersed in liquid nitrogen (-196 °C) for storage.

#### *Evaluation of sperm motility*

Sperm motility was assessed with the CASA module (NIS Elements Ar 3.2.), using the camera JENOPTIK ProGres CT1 (30 fps) and stereo microscope (Nikon Eclipse E600) with heated plate (Tokai Hit). A 3  $\mu$ l volume of the sample was evaluated in a calibrated counting chamber Leja® (depth 20  $\mu$ m) in 6 fields per one sample. The native ejaculate samples as well as the freeze-thawed ones were assessed after 5-minute tempering (“0 hour”) and 2-hour incubation in a water bath at 37 °C. In both cases 3 selected motility parameters were assessed - curvilinear motility (VCL,  $\mu$ m/s) average path velocity (VAP,  $\mu$ m/s) and straight line velocity (VSL  $\mu$ m/s).

#### *Statistical analysis*

Statistical analysis was performed with STATISTICA CZ 10, multi-factorial ANOVA method and subsequent Scheffe’s post-hoc test was used.

### **Results and discussion**

In our study we tested the effect of LDL addition to the composition of a commercially manufactured plant phospholipid-based extender in proportional ratios 4%, 6% and 8%. The values of monitored motility parameters VCL, VAP, and VSL were significantly higher ( $P < 0,05$ ) at the LDL enriched samples in comparison with the control numbers of samples diluted in pure AndroMed® (Minitübe, Germany) under our conditions. This effect was determined in diluted native semen as well as in freeze-thawed samples (Table 1 and 2).

No statistically significant differences across the concentration range of added LDL were found. The values of monitored parameters of post-thaw motility obtained in our study were lower in contrast to the studies by Moussa et al. (2002), Amirat et al., (2004), or Amirat et al. (2005). This difference could be related to the different length of the incubation period or can be associated with prolonged exposure to BSP proteins before semen processing, as these can have a negative effect on sperm motility (Manjunath et al., 2002; Bergeron et al., 2004). Differences may also be related to the fact that cryopreservation process was rather simplified under laboratory conditions without the appropriate freezer. However, in spite of this fact, positive effects of LDL fraction addition were conclusive. Due to the lack of studies on LDL effects dealing with sperm motility before freezing, it is difficult to comment this part of our experiment. Nevertheless, we can say that the LDL positive effect was found even before the freezing-thawing process.

Table 1

Sperm movement characteristics of bull native ejaculate extended with the commercial plant phospholipid-based extender Andromed with different portion of egg-yolk LDL and incubated at 37 °C (mean ± SD)

Extender	0% LDL		4% LDL		6% LDL		8% LDL	
	0 hour	2 hours	0 hour	2 hours	0 hour	2 hours	0 hour	2 hours
VCL (µm/s)	102,1 ± 0,917 <sup>ac1</sup>	53,7 ± 0,846 <sup>ABC1</sup>	114,1 ± 0,863 <sup>ab2</sup>	91,9 ± 0,823 <sup>A2</sup>	104,3 ± 0,882 <sup>b</sup>	97,2 ± 0,824 <sup>B</sup>	108,7 ± 0,869 <sup>c4</sup>	94,8 ± 0,833 <sup>C4</sup>
VAP (µm/s)	54,1 ± 0,492 <sup>a1</sup>	26,3 ± 0,453 <sup>ABC1</sup>	60,1 ± 0,463 <sup>a2</sup>	48,9 ± 0,441 <sup>A2</sup>	56,5 ± 0,473 <sup>3</sup>	50,1 ± 0,442 <sup>B3</sup>	59,0 ± 0,466 <sup>4</sup>	48,1 ± 0,467 <sup>C4</sup>
VSL (µm/s)	43,7 ± 0,461 <sup>1</sup>	20,8 ± 0,425 <sup>ABC1</sup>	47,8 ± 0,434 <sup>2</sup>	39,2 ± 0,414 <sup>A2</sup>	45,2 ± 0,444 <sup>3</sup>	39,5 ± 0,414 <sup>B3</sup>	47,2 ± 0,437 <sup>4</sup>	36,9 ± 0,419 <sup>C4</sup>

a, A, 1 -values in the raw signed with the same superscript differ significantly at P < 0,05

Table 2

Sperm movement characteristics after freeze-thawing processing of bull ejaculate extended with the commercial plant phospholipid-based extender Andromed with different portion of egg-yolk LDL and incubated at 37 °C (mean ± SD)

Extender	0% LDL		4% LDL		6% LDL		8% LDL	
	0 hour	2 hours	0 hour	2 hours	0 hour	2 hours	0 hour	2 hours
VCL (µm/s)	33,0 ± 0,649 <sup>abc</sup>	30,5 ± 0,639 <sup>ABC</sup>	47,9 ± 0,642 <sup>a2</sup>	37,1 ± 0,625 <sup>A2</sup>	46,1 ± 0,646 <sup>b</sup>	41,8 ± 0,605 <sup>B</sup>	47,6 ± 0,641 <sup>c4</sup>	37,6 ± 0,645 <sup>C4</sup>
VAP (µm/s)	17,2 ± 0,341 <sup>abc</sup>	14,4 ± 0,336 <sup>ABC</sup>	24,7 ± 0,337 <sup>a2</sup>	17,8 ± 0,328 <sup>A2</sup>	24,1 ± 0,191 <sup>b3</sup>	20,2 ± 0,318 <sup>B3</sup>	25,3 ± 0,340 <sup>c4</sup>	18,0 ± 0,339 <sup>C4</sup>
VSL (µm/s)	14,5 ± 0,320 <sup>abc1</sup>	11,5 ± 0,315 <sup>ABC1</sup>	20,1 ± 0,317 <sup>a2</sup>	14,5 ± 0,308 <sup>A2</sup>	19,8 ± 0,319 <sup>b3</sup>	16,3 ± 0,298 <sup>B3</sup>	21,0 ± 0,316 <sup>c4</sup>	14,4 ± 0,318 <sup>C4</sup>

a, A, 1 -values in the raw signed with the same superscript differ significantly at P < 0,05

The cryoprotective properties of LDL were verified mainly in studies using LDL as a substitute for the yolk in egg-yolk-based extenders (Moussa et al., 2002; Amirat et al., 2004; Amirat et al., 2005; Amirat -Briand et al., 2010; Hu et al., 2010; Hu et al., 2011). Those papers usually concluded, through testing the post-thaw effect of different concentrations, that extender with 8% of LDL is optimal and even better than control egg-yolk-extender. These effects were gradually confirmed not only on the basis of sperm motility evaluation but also by analysis of other sperm cells quality indicators. These findings are in accordance with results of Pace and Graham (1974) who found a detrimental effect of other components present in egg yolk on sperm motility. Also the proven optimal concentration is noteworthy because egg yolk naturally contains 7% LDL, thus very close to the experimentally defined optimum.

Plant phospholipid-based extenders Biociphos or Bioxcell (both - IMV, L'Aigle, France) were included in the studies by Moussa et al. (2002), Amirat et al. (2005) and Vera-Munoz et al. (2009), but only as control samples. The results showed no significant difference compared with egg-yolk extenders in which the yolk was replaced by LDL fraction. But the effect of LDL addition to the plant phospholipid-based extenders has not been tested yet. With regard to phospholipid content in these extenders, without any chance to remove it from

composition, we chose besides the recommended 8% LDL concentration also lower ones (4%, 6%) within our study. It is noteworthy that under our experimental conditions even these lower concentrations of LDL significantly increased the level of monitored parameters of sperm motility in the case of native ejaculate and post-thawed as well. Although we did not prove any significant differences in effect among LDL concentrations tested, slightly better results could be observed at 6 % LDL.

The positive effect of LDL on sperm motility, found also in our study, can be explained by more properties of this fraction: Hu et al. (2011) demonstrated a positive effect of LDL on sperm motility by supporting the activities of glutathione peroxidase (GSH – Px), reduced glutathione (GSH) and catalase (CAT) which are part of the sperm antioxidative system (Aitken and Baker, 2004). Furthermore, as shown by Manjunath et al. (2002) and Bergeron et al. (2004) LDL has an ability to form very stable complexes with the major bovine seminal plasma proteins - BSP proteins (BSP - A1 / A BSP - A3 BSP -30 kDa). These mentioned proteins can stimulate the efflux of cholesterol and choline phospholipids from the plasmatic membrane, thereby subsequently influencing on membrane integrity. Simultaneously an increase in lipid content in the plasma membrane was also demonstrated and therefore the possibility of incorporation of part or of whole LDL molecules into this sperm cell structure. The dose-independent effect of even low LDL level observed in our study might be related to a simple increase of general phospholipid content to the maximum effective level as well as to a combination of egg-yolk LDL and plant phospholipid properties.

### **Conclusion**

We found a positive influence of egg yolk LDL fraction addition to a plant phospholipid-based extender on sperm motility before cryopreservation as well as after cryopreservation and subsequent thawing. This beneficial effect was demonstrated in all selected LDL concentrations. There is a need, however, for further studies to assess the optimal concentration in plant phospholipid-based extenders and also to verify the LDL interaction with various kinds of these extenders. Further studies are also needed to elucidate the precise mechanisms of the LDL effect.

### **Acknowledgment**

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## SCORING SYSTEM AND IDENTIFICATION OF MASTITIS RISKS IN CATTLE FARMS

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### Summary

Farm management failure is considered to be the cause of much sub-clinical prevalent pathologies in dairy cattle rearing. The aim of this study was: the quantitative evaluation of data assessment in farm management through the use of institutional instruments such as questionnaire and system of points (Scoring system) and their comparison with the laboratory analyses of milk, in a “X” farm situated in Ndroq, Tirana. The intention was to rank critical points during cattle breeding and their redress. For these purposes were conducted two surveys, by 8 months time frame, according to literature consulted, associated with quantitative evaluation of farm husbandry practices and milk samples. The breeding-quantitative quotation (milking, lactation, calving, etc...) was performed by point evaluation of specific practices. The smallest score point was 1 and the biggest was considered from 4 to 10.

For each file were calculated the total points and then the average score. According to LS (Linear Score), maximal average number of points for each file should not exceed 4.5 to 4.9 points. At this point level Somatic Cells are 300-400 for SG 000/milk ml (Zecconi 2010).

Total aerobic and somatic cells were analyzed as raw milk indicators.

In the first survey the results for somatic cell were:  $580 \pm 50$  for  $10^3$ /ml and for total aerobic cells were:  $1430 \pm 157$  for CFU (Colony Forming Units) / milk ml. At the second survey the somatic cells were  $290 \pm 40$  while total aerobics  $138 \pm 58$ , versus 400 and  $<1000$  that are mark respective rates, due this to rearing management corrections, which reflect directly to average scores.

During this study it was concluded that the most critical point during milking was the teat disinfection (before and after milking), practices which were not carried out correctly or were performed by inefficient pharmaceutical substances. Our suggestions that are in concordance with the farmers association, led to the correction of many farming practices, where the most indicative was the decreasing by 10 points to the score 1 point in milking file schedule during the second survey, which was also reflected in hygienic status of milk.

**Key words:** Udder health, Somatic cells, Total Aerobic indicators, Milking.

### Introduction

The milk quality and quantity production is related to a correct functioning of the udder and optimized health status of the organism. Mastitis is not just a serious problem for animal health, but presents also a serious threat to human public health and is considered one of the main causes of economic losses. Farm management deficiencies are currently considered as the origin of this disease with less obvious symptoms, such as subclinical mastitis, which has become dominant in cattle breeding (Zecconi A. et al 2006; Sol, J et al 2000).

An accurate identification of the "critical points" in the farm by the implementation of HACCP procedure is essential and represents the basis of a healthy farm. The veterinarian interventions are imperative and require the availability of study objectives and practical tools for assessing to the proper farm management. The use of severe instruments such as questionnaires and pointing systems (Scoring system) for the evaluation of farm management, promise an objective assessment of herd health status and facilitate the veterinarian addressing toward critical points during livestock breeding (Zecconi.A, 2010).

### **Purpose of the study**

The purpose of this study is: to estimate the relationship among the farming practices point scoring and the infection presence (sub-clinical mastitis YES/NO), in a study conducted in the farm "X", at Ndroq – Tirana.

Aim of this study is: the identification of critical points in cattle farming, through the use of institutional instruments such as questionnaire and pointing systems (Scoring system).

### **Materials and methods**

The study was conducted on the dairy cattle farm "X", in Ndroq – Tirana.

Analyses were performed in the laboratories of Albanian Milk and Meat Association “ADAM”; Veterinarian Public Health Department of Faculty of Veterinary Medicine, Tirana - Albania and Infectious Diseases Department, Faculty of Veterinary Medicine, Milan – Italy.

#### **1. Questionnaire and data collection**

Goodger et al. (1993) and Zecconi (2010) have developed a list of indicators (that makes the questionnaire) easily monitored for assessing to the farm management practices.

Initially, the questionnaire based on breeding conditions of the farm was adapted. The data were collected at the farm by completing the questionnaire and farm visiting. The questionnaire covers all aspects of farm management, with greater emphasis on the activities related to general health management and in particular to udder health status (this case study).

#### **2. Cytological and bacteriological analyzes of milk in the farm**

##### **Type of sample to analyze**

In this study was analyzed the raw tank milk, in order to identify the presence of sub-clinical mastitis. The samples are usually taken for the milk quality assessment and the health status of the herd. Also, the milk of all quarters was analyzed in order to determine prevalence of mastitis etiological agents (Fox L. et al 2003; Zecconi. A, 2010).

To obtain accurate results sampling was much cautioned: the milk for sampling was previously mixed in the tanks and sterile test tubes were used. Samples were preserved at 4<sup>0</sup>C and so transported to the laboratory within 24 hours (Zecconi. A, 2004).

**The analyzed indicators** (somatic cells in milk, total aerobics in milk, pH, Proteins, Fat):

To define the most prevalent mastitis pathogens, were analyzed 96 milk samples prevailed from 24 cows (in small capacity farms with fewer than 40 dairy cows normally are sampled 15 heads, in farms with more than 40 dairy cows are sampled 24 heads).

Assignment of sampled cows was performed on the basis of days of lactation as follows: 50% > 200 days; 30% <30 days; and 20% in the interval between 30 and 200 days of lactation (Zecconi.A, 2010).

### 3. Point evaluation system (Scoring System)

To each farm management practice, during this study was attributed a point system: the more correct a practice was performed and managed the lower was the score number of points attributed to it.

The smallest score number is 1 and the largest number is considered a score number from 4 to 1, depending on practice scheduled. The accuracy is determined by reference practices and consulting expert opinion.

The score point value for each data was calculated by the sum of every schedule voice. For each file was computed the total and the average of resulted points.

The obtained data are normally correlated to the content of somatic cells in milk (Zecconi 2010).

### Results & discussions

The udder health and quality of milk are primarily assessed by the presence of somatic cells in it, as it is the surveyed indicator of this study.

For raw tank milk, the limit of 200.000 to 400.000 somatic cells/ml is an awareness value and not a critical threshold as in animal milk (dairy cow).

A normal and healthy situation of the farm management is numbered from 200,000 to 300,000 somatic cells/ ml raw tank milk (Zecconi 2010).

Table no. 1. Cytological and bacteriological milk indicators

Indicators Timeline	Somatic cells/000/ milk ml		Total Aerobics /000 cfu/ milk ml	
	Results	Norm (Regulation)	Result	Norm (Regulation)
<b>FIRST SURVEY</b>	<b>580 ± 50</b>	<b>400</b>	<b>1430 ± 157</b>	<b>&lt;1000</b>
<b>Two Months after the First Survey</b>	<b>560 ± 43</b>	<b>400</b>		
<b>Four Months after the First Survey</b>	<b>450 ± 40</b>	<b>400</b>		
<b>Six Months after the First Survey</b>	<b>310 ± 25</b>	<b>400</b>		
<b>SECOND SURVEY</b>	<b>290 ± 40</b>	<b>400</b>	<b>138 ± 58</b>	<b>&lt;1000</b>

As it is shown in on Table no. 1 the differences in the content of somatic cells and total aerobics in milk, are very sensitive to the parallel monitoring during the first interview comparing to the second monitoring. In the first survey these indicators are at upper norm so they attest for mastitis presence. In the second survey, after our intervention to the management practices (which is expressed in point scoring too) the values are within the norm, even quite reduced (Fox L. et al 2003; Zecconi 2010).

Our somatic cell monitoring was performed every 2 months, which brought up to their gradual decrease up to 290,000 somatic cells / milk /ml.

Table no. 2. Milk chemical indicators

Indicators Timeline	pH		PROTEINS %		FAT%	
	Result	Standart	Result	Standart	Result	Standart
<b>FIRST INTERVIEW</b>	<b>7.0</b> <i>increased</i>	<b>6.6-6.7</b>	<b>3.8</b> <i>increased</i>	<b>3.2</b>	<b>3.3</b> <i>decreased</i>	<b>3.8-4.2</b>
<b>SECOND INTERVIEW</b>	<b>6.66</b>	<b>6.6-6.7</b>	<b>3.3</b>	<b>3.2</b>	<b>4.1</b>	<b>3.8-4.2</b>

Table no. 2 reports that even the milk chemical indicators were not at normal levels during the first parallel monitoring and interview. The levels of proteins and pH resulted increased in value, meanwhile fat levels were decreased, and such results are in concordance with previous studies data (Zecconi A. et al, 2006).

The daily milk production at the first interview per dairy cow with mastitis was 17 liters, whereas at the second interview, after the milk chemical indicators were rated to normal rates, was 20 liters. Even this data is consistent to the consulted references, which indicate for a 15% daily milk decrease production per head.

Bacteriological test results reported the presence of mastitis pathogens as: *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus agalactie* (Table no. 3)

Subclinical mastitis prevalence in total sampled animals resulted 37.5% respectively: 20.8% for *Staphylococcus aureus*, *Streptococcus agalactie* for 5.2% and 7.3% for *Escherichia coli*.

Table no. 3. Bacteriological results of milk samples

Isolated microorganisms	Number of Positive Samples	%	Number of Negative Samples	%
<i>Staphylococcus aureus</i>	21	20.8	75	79.2
<i>Streptococcus agalactie</i>	5	5.2	91	94.8
<i>Escherichia coli</i>	7	7.3	89	92.7

Referring to the score evaluation our results it could be concluded that the most important issue is dairy cattle milking with an average sum of 5.4 points and then followed by lactation, cattle drying and heifers (situated within a unique farm) with 5 points score (Zecconi A. et al 2006; Sol, J et al 2000).

Referring to **Linear Score**, recommended by Zecconi 2010, a milking schedule with 5.5 points score corresponds to a number of 560.000 somatic cells/milk ml, which complies with milk analysis results in parallel of the first survey (number of somatic cells per raw tank milk ml resulted 580.000 cells).

Also the result of 5 points score of lactation, cattle drying and heifers (situated within a unique farm), are a critical control point in terms of this score correlation with the content of somatic cells / raw tank milk ml.

Table no. 5. Point scoring of farm management practices

<i>Schedules/Files</i>	SCORE POINTS			
	FIRST SURVEY		SECOND SURVEY	
	TOTAL	AVERAGE	TOTAL	AVERAGE
<b>AVERAGE SUM RESULT</b>	<b>20</b>	<b>2.5</b>	<b>9</b>	<b>1.1</b>
<b>LACTATION</b>	<b>75</b>	<b>5 !!</b>	<b>67</b>	<b>4.4!!</b>
<b>CATTLE LACTATION DRYING</b>	<b>75</b>	<b>5</b>	<b>67</b>	<b>4.4</b>
<b>HEFERS</b>	<b>75</b>	<b>5</b>	<b>67</b>	<b>4.4</b>
<b>MILKING</b>	<b>141</b>	<b>5.5 !!</b>	<b>64</b>	<b>2.4!!</b>
<b>PARTURITION</b>	<b>80</b>	<b>4.7</b>	<b>68</b>	<b>4</b>
<b>THERAPY</b>	<b>43</b>	<b>2.7</b>	<b>43</b>	<b>2.7</b>
<b>SUM</b>	<b>509</b>	<b>30.4</b>	<b>385</b>	<b>23.4</b>

Farm management interventions, specifically improvements in milking practices management (which is considered a critical point for the mastitis presence), and also somewhat lactation, lactation drying and heifer, significantly reduce the number of points assessed in scheduled questionnaires during the second survey, which is consistent with milk analyzes results (the number of **somatic cells / raw tank milk ml**) proceeded parallel with the second survey.

### Conclusions

- Quantitative assessment of breeding practices enables the farm auto control.
- The study confirmed a positive correlation between score pointing of husbandry practices and hygienic status of milk.
- Maintaining a constant point score and comparing it to the Linear Score, in key practices as milking, lactation, heifer, etc. ensures hygienic quality of milk.
- Milking is the most important critical point in dairy cattle where specifically is remarked the nipples before milking preparation and their before and after milking disinfection.

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**THE INFLUENCE OF DIFFERENT INTENSITIES OF FEEDING ON THE GROWTH OF JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WAL.)**

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**Abstract**

The paper presents the results of the research influence of different intensities of feeding on the growth of juvenile rainbow trout (*Oncorhynchus mykiss* Wal.) with an average initial weight and body length ( $\pm$ SD) 2.71 $\pm$ 0.87 g - 6.30 $\pm$ 0.72 cm, 2.55 $\pm$ 0.75 g - 6.18 $\pm$ 0.58 cm and 2.66 $\pm$ 0.82 g - 6.32 $\pm$ 0.60 cm. The experiment is realized in the laboratory of Fisheries, Faculty of Agriculture in Banja Luka. The experimental rainbow trout fingerlings divided into three groups; feeding every day (EXPG<sub>C</sub>), a group without feeding on Sunday (EXPG<sub>Sun</sub>) and the group without feeding on Wednesdays and Sundays (EXPG<sub>Wed-Sun</sub>). After 42 days of average weight and body length ( $\pm$ SD) was in EXPG<sub>C</sub> 9.93 $\pm$ 3.85 g - 9.60 $\pm$ 1.26 cm, EXPG<sub>Sun</sub> 8.95 $\pm$ 2.82 g - 9.29 $\pm$ 0.95 cm and EXPG<sub>Wed-Sun</sub> 7.96 $\pm$ 2.79 g - 8.97 $\pm$ 1:00 cm. Significant differences ( $P < 0.05$ ), average body weight were found between the experimental group EXPG<sub>C</sub> and EXPG<sub>Wed-Sun</sub>. Specific growth ratio (SGR) and thermal-unit growth coefficient (TGC) have a tendency to decrease in proportion food intake, and feed conversion ratio (FCR) has the reverse trend, with the lowest EXPG<sub>Wed-Sun</sub>, followed EXPG<sub>Sun</sub> and EXPG<sub>C</sub>.

**Key words:** rainbow trout, feeding intensity, growth

**Introduction**

Feeding farmed rainbow trout, one of the most important coldwater fish that is cultivated in a number of countries in the world, occupies a significant place in technology and growing participation in the cost of production.

Salmonid fish species inhabit the clean and clear water, so it is grown in conditions necessary to ensure an adequate supply fish farms of first-class water quality (Mitrovic-Tutundžic and Brkovic-Popovic, 1994, Kahrmanovic et al., 2013, Savic et al., 2013 a, b).

Intensification of cultivation salmonid fish, which included rainbow trout (*Oncorhynchus mykiss* Wal.), natural food has lost its significance due to the large number of individuals per unit of production volume, and nutrition using pelleted and extruded food that provides all the needs of farmed fish (Markovic and Mitrovic-Tutundžic, 2003).

The importance of continued research feeding farmed rainbow trout is large, given that the share of food expenditures in total production costs by 50 to 60%. The continuous development of food is important and in order to achieve optimal growth characteristics that are directly affected by environmental conditions, quality and health status of young rainbow trout, food quality and food technology

The aim of this study was to analyze the effects of feeding different intensity on the growth of juvenile rainbow trout (*Oncorhynchus mykiss* Wal.).

## Materials and methods

The experiment was carried out for a period of 42 days (22.2.2013.-06.4.2013.) in experimental polygon at Faculty of Agriculture Banja Luka. Rainbow trout is the populated in the 55 l aquarium with 42 fish in each experimental group.

Water temperature (°C), content of dissolved oxygen (mg/l) and water oxygen saturation (%) were analyzed by digital oxy-meter Oxi 330i/SET 2b20-0011 WTW, and the pH value of a digital pH-meter pH 330i/SET WTW 2A20-1011 (Germany), every day before feeding.

During the experimental period were followed characteristic growth of body mass (the scale Denver DL-501 payload 0.5 kg) and body length (ichtyo-meter). Useful volume of water in aquarium was 55 l/aquarium with a constant flow of fresh water in all aquariums independently.

In the experiment was used extruded trout feed with 52% crude protein, 20% crude fat and 19,6 MJ/kg digestible energy, and fish were fed twice daily, at 9 and 15 o'clock.

Mass and body length were determined on a sample of 42 fish/exp group. On the basis of absolute indicators of mass and body length were calculated condition factor, specific growth rates and thermal-unit growth coefficient. Condition factor (CF) was calculated according to the formula:  $CF = (BW/L^3) \times 100$ , where CF - condition factor; BW – body weight (g); L - length of fish (mm). A specific growth rate was calculated according to the formula:  $SGR = ((\ln FBW - \ln IBW) / D) \times 100$ , where FBW - final body weight (g), IBW - initial body weight (g), ln - natural logarithm; D-days. Thermal-unit growth coefficient is calculated according to the formula:  $TGC = [FBW^{1/3} - IBW^{1/3}] / \sum [TxD] \times 100$ , where TGC-thermal-unit growth coefficient; FBW - final body weight (g), IBW - initial body weight (g), T - temperature (°C), D - days. Feed conversion ratio (FCR) was calculated according to the formula:  $FCR = F/G$ , where the F-food consumption, G-realized weight gain.

Statistical analysis of the data included descriptive statistics (mean value, standard deviation and coefficient of variation), a simple analysis of variance and t - test (Microsoft Office Excel 2003: Statistical Analysis - ANOVA).

## Results and discussion

During the experimental period, daily water quality was analyzed on the basic parameters of the physical and chemical characteristics of water (water temperature, dissolved oxygen content in water saturation of water with oxygen and pH) are important for fish farming (Table 1).

The results of most of the analyzed physical and chemical characteristics of water shown in table 1 indicate on the optimal values for the cultivation of young rainbow trout. The exception is the saturation of water with oxygen, which is in all aquariums averaged less than 90% (from 85.43 to 87.77%).

Although during the experiment, compared to the optimal (90-100%), showed a slightly lower water oxygen saturation, were recorded good growth characteristics in all analyzed treatments. Generally, the variation of the analyzed characteristics of water in all treatments does not indicate significant differences between treatments as suggested by the coefficient of variation for each analyzed trait.

Consequently, we can say that the quality of water in all treatments was similar with the exception of water saturation as indicated.



Table 1 Results of the analysis of physical and chemical properties of water throughout the experimental period (water temperature, dissolved oxygen, oxygen saturation of water and pH value)

Treatmans	Water temperature, °C			Dissolved oxygen (O <sub>2</sub> ), mg/l			Oxygen saturation of water (O <sub>2</sub> ), %			pH		
	Mean values±SD	min-max	CV	Mean values±SD	min-max	CV	Mean values±SD	min-max	CV	Mean values±SD	min-max	CV
EXPG <sub>C</sub>	12.88±0.34	12.2-13.8	2.64	9.09±0.30	8.53-9.61	3.29	87.77±2.86	82.1-91.8	3.26	7.39±0.15	7.14-7.92	2.08
EXPG <sub>Sun</sub>	12.88±0.29	12.3-13.3	2.22	8.81±0.32	8.38-9.80	3.57	85.43±2.75	80.2-93.8	3.22	7.25±0.10	7.07-7.58	1.44
EXPG <sub>Wed-Sun</sub>	12.87±0.31	12.3-13.4	2.41	8.91±0.32	8.31-9.68	3.59	86.27±2.60	81.3-92.6	3.02	7.28±0.09	7.05-7.49	1.25

In the table 2 are shown the growth characteristics of juvenile rainbow trout (*Oncorhynchus mykiss* Wal.) from three treatments with different feeding intensity.

Table 2. Growth parameters of experimental groups rainbow trout

Parameter	Treatments - Experimental group (n = 42)		
	EXPG <sub>Control</sub>	EXPG <sub>Sun</sub>	EXPG <sub>Wed-Sun</sub>
<b>Initial</b>			
W±SD (g)	2.71±0.87 <sup>ns</sup>	2.55±0.75 <sup>ns</sup>	2.66±0.82 <sup>ns</sup>
CV <sub>W</sub>	31.95	29.48	30.92
L±SD (cm)	6.30±0.72 <sup>ns</sup>	6.18±0.58 <sup>ns</sup>	6.32±0.60 <sup>ns</sup>
CV <sub>L</sub>	11.39	9.45	9.57
CF	1.084	1.080	1.054
Feeding days	41	36	31
<b>Final</b>			
W±SD (g)	9.93±3.85*	8.95±2.82 <sup>ns</sup>	7.96±2.79*
CV <sub>W</sub>	38.8	31.48	35.02
L±SD (cm)	9.60±1.26	9.29±0.95	8.97±1.00
CV <sub>L</sub>	13.18	10.27	11.17
WG (g)	7.22	6.40	5.30
LG (cm)	3.29	3.11	2.65
Survival (%)	95.24	95.24	100.00
FCR	0.64	0.63	0.61
CF	1.122	1.116	1.103
SGR	3.092	2.989	2.610
TGC	0.144	0.135	0.116

W – body weight (g); CV<sub>W</sub> – coefficient variation of body weight; L – body length (cm); CV<sub>L</sub> – coefficient variation of body length; WG - weight gain (g); LG - length gain (cm); FCR – feed conversion ratio; FE – feed efficiency; CF - condition factor; SGR – specific growth ratio; TGC – thermal-unit growth coefficient. <sup>ns</sup> not significantly; \* p<0.05

Beginning weight and body length of young rainbow trout in all treatments was similar (no statistically significant difference), with the coefficients of variation of body weight significantly expressed. At the end of the experiment was statistically significant difference in mean of body weight (p <0.05) between treatments EXPG<sub>Control</sub> and EXPG<sub>Wed-Sun</sub>, and there was no statistically significant differences in mean values body length in any combination. Increase of body weight expressed are high coefficients of variation and of the body weight, particularly in treatments between which significant difference in (EXPG<sub>Control</sub> and EXPG<sub>Wed-Sun</sub>). Coefficients of variation of body length at the beginning and the end of the experiment were similar. The coefficient of condition at the end of the experiment compared to the initial is increasing at all treatments, indicating that although the treatment EXPG<sub>Sun</sub> fish is not fed one day during the week, and in the treatment of EXPG<sub>Wed-Sun</sub> fish is not fed 2 days during the week, and the ratio of growth of the body length is increasing in the required limits when it comes to condition.

Specific growth ratio (SGR) and thermal-unit growth coefficient (TGC) have a tendency to decrease in proportion food intake, and feed conversion ratio (FCR) has the reverse trend, with the lowest EXPG<sub>Wed-Sun</sub>, followed EXPG<sub>Sun</sub> and EXPG<sub>Control</sub>. In the experimental group EXPG<sub>Wed-Sun</sub>, in which the fish is not fed twice per week, the lowest FCR and the best utilization of food, but the increase in body mass is proportional nutrition.

The found FCR (0.64; 0.63 and 0.61) agree with the results Choobkar (2008), which states that the FCR of rainbow trout average weight of about 10 g at different feeding frequencies (4, 6 and 8 times) 0.61 ; 0.51 and 0.52, while the SGR identified in this study was significantly lower (3.092; 2.989; 2.610) compared to the results of the aforementioned authors (3.78; 4.19, 4.12). Also, CF in the according to the intensity of feeding decreased the of  $EXPG_{Control}$  to  $EXPG_{Wed-Sun}$  group and agrees to the Choobkar (2008), which states that the CF at different frequency of feeding ranges from 1.05 to 1.24 and allegations Karabulut et al (2010) to CF in water temperatures of 9 and 15°C and different feeding around 1.7 and similar results Uysal and Albaz (2002) and Savic et al. (2012).

Reducing the number of feeding days FCR is lower according Choobkar (2008) and contrary to the allegations Guzel and Arvas (2011), who argued that the control group had the lowest FCR, and the reduction in the number of feeding days FCR is increasing, while the CF in rainbow trout average weight of about 10 g significantly higher (1.32 to 1.38) compared to the results presented in this paper.

Mortality during the experimental period was 4.76% (2 individuals) in the treatment of  $EXPG_{Control}$ , treatment  $EXPG_{Wed-Sun}$  also was 4.76% (2 individuals) was similar to the Karabulut et al. (2010), while in treatment  $EXPG_{Wed-Sun}$  no mortality.

### Conclusion

The growth patterns of young rainbow trout (*Oncorhynchus mykiss* Wal) CF, SGR and TGC agree feeding intensity, the highest growth indicators were found in the experimental group  $EXPG_{Control}$ , and lowest in  $EXPG_{Wed-Sun}$ . Also, in the experimental group  $EXPG_{Control}$  were recorded the highest coefficients of variation, particularly of body weight. At the same time, the lowest FCR is in the experimental group  $EXPG_{Wed-Sun}$  which indicates a longer retention of food in the digestive tract and a higher degree of utilization of nutrients. Although marked differences in average mean weight and body length in all three treatments was statistically significant mean difference in average body weight only between the treatments  $EXPG_{Control}$  and  $EXPG_{Wed-Sun}$ , while the other combinations are not the significant differences.

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## THE SITUATION OF PRODUCTION AND PURCHASE OF RAW MILK IN BOSNIA AND HERZEGOVINA

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### Abstract

This paper analyzes the production of milk as one of the most important branches of agriculture, which has been and should remain the backbone of its further development.

The structure of agricultural production in Bosnia and Herzegovina is unfavorable. Share of livestock production in the value of total agricultural production is about 40%. The main problems that face livestock and animal production in Bosnia and Herzegovina are small production, excessive imports, unfavorable structure of production, and low price of milk purchased and a very low standard of producers who are on the edge of existence.

Milk production in BiH, unfortunately, is still underdeveloped, disorganized and often does not meet the needs of industrial dairy processing capacity. Only 5% of milk producers with produced the quantity and quality of milk is fitting with their results in the requirements of market economy. Larger quantities of produced cow's milk do not meet the criteria of quality, particularly hygienic criteria in accordance with the requirements of international standards and quality regulations. Milk processing (and the market) and the consumption of milk and dairy products is the consequence of the unfavorable economic situation, decreasing living standards and purchasing power, and insufficient education of consumers about the importance of milk and milk products in the daily diet.

Dominant production system are small farms (1-3 cows) dealing with mixed livestock production, with the primary goal of self-sufficiency.

The data used in the paper were derived from statistical agencies, as well as his own research in the 6 largest milk processors in BiH (purchase and processing about 85% of total milk purchased in BiH). Number of dairy farmers of these 6 is 15,311, who own a total of 42,364 dairy cows. Average per cooperant is 2.77 cows. Average milk production per dairy cow is 4.081 liter.

Milk quality in accordance with Milk quality decree is unsatisfactory. E-class quality of the milk is only 42%, and worrying data is constant increase the milk quantity which, in accordance with the Decree, puts the raw milk outside of the class (2012th year 26%).

**Keywords:** milk, purchase, production

### Introduction

The total level of development of agricultural production in a country depends, among other things, of the participation of livestock production in the structure of agriculture. Within livestock production milk production in Bosnia and Herzegovina is one of the most important branches of agriculture

The conditions for livestock production, from the standpoint of providing the volume and quality of feed are favorable. Changes in the structure of sowing areas and increased yields, can provide greater forage crops production. In the production of forage and concentrate feed positive changes can be made which represents a real difference that present a solid foundation for the growth of livestock production.

In developed market economies the main motive for milk production has become a profit. Milk production is affected by agro climatic factors, the level of economic development, religious composition of the population and the traditional aspect of the habit of consuming milk and milk products. The breed of cattle, measures of selection and production technology will influence level of productivity, quality of cow milk and production profitability.

Milk and milk products are an economic activity in agriculture and food processing dairy industry that is using milk as a raw material.

Bosnia and Herzegovina is one of the few European countries that, despite of favourable conditions for cattle raising, does not produce enough milk for their needs. Therefore, the strategic goal in milk production should be better use of comparative advantages and existing resources in order to achieve sufficient production for the domestic market. Here it should be noted that self-sufficiency is not a strategic objective, but consequence of better usage of existing comparative advantages in the milk production.

Rural households with poor land and capital, low production with a small number of cows per farm prevail. Development needs are imposing farm enlargement and construction of new, modern dairy farm. Increase of milk production on farms requires substantial capital. If the increase of households depends of the farm financial success and their own investments with the current conditions, milk production will continue to decline.

While government policy is inefficient, it cannot be expected that effect of economic principles encourage an increase in milk production. The world beyond recognition and globalization requires a shift that will properly evaluate and appreciate each farmer and each cow, a change that will encourage farmers to continue to cultivate the land, cherish rural environment and supply us with quality local products.

Especially in conditions of the extensive milk production there is a danger and the risk of inadequate hygienic quality of milk in relation to the number of microorganisms and somatic cells, the content of allowable residues of antibiotics, hormones and other banned substances. Therefore fresh raw cow's milk, as commercial milk that has been delivered to markets for further processing and use, must comply with the conditions prescribed by the international quality standards.

The European Union adopted in 1992. Directive on the conditions of production and sale of raw milk, processed milk and dairy products (Directive 46/92., Milk and milk product quality, health and hygiene).

In practice among EU member states there are some differences in the application of rules on hygienic correctness, but only in relation to the method of calculation and payment of milk.

The use and consumption of milk depends on the level of development of a country, purchasing power, food habits and standard of the population. In developed countries, more than 90% of milk produced is sold to dairies. In underdeveloped countries, productivity is at a low level while the milk is being used mainly for human consumption in households or in part, through dairy products sold in the local markets. Developed countries from domestic production of milk and milk products provide total annual demand and the surplus is exported. The international trade in milk and dairy products may include only those manufacturers that provide a certain assortment and the famous brand, and the ones that in the fierce competition can offer more favorable purchase price.

Level of profit is the main growth factor of milk production per cow and increase of dairy farms. An increase of milk production per cow and increase of dairy farms has emerged a number of new relations in the field of cattle and land relations, relations of production and consumption of milk.

Characteristics of milk production in Bosnia and Herzegovina can be linked to the conclusion of milk production in Croatia - Most economies function as acquired social category, not a business system, beef production is their tradition, a way of life, and less business activity.

There is an obvious low level of utilization of the production potential per cow, usually due to improper feeding and accommodation. Breed selection as the means of production is not profiled and it is inappropriate for possible intensity of production (Caput, 2003).

According to research by the Expert Centre for farm management and knowledge transfer, and the Institute of Animal Science, Research Center of Wageningen in the Netherlands (Grip, 2003), the order of importance in managing the farm is as follows: the most important is nutrition (3.1), and right behind it is farm economics (3.5), followed by grassland management (3.8) and animal health (3.9). In the middle of importance is milking (5.1). The following is management of minerals (6.0), growing offspring (6.3), the organization of labor (6.8), breeding and selection (7.1) and machinery (9.2). In this graduation, a 1.0 rating is the most important in the management, while the score 10 least significant.

### Materials and methods

On the basis of available statistical data and calculations, this paper shows the number of dairy cows that is the basis for the production of milk in the period in 2002. to 2012th in the Federation of Bosnia and Herzegovina and the Republic of Srpska. Then it is showed the volume of milk production and milk yield per dairy cow, analysis of milk purchase, and the number of milk producers and the average production per producer in the 2012th year; for this data we used survey of 6 largest dairies in BiH that purchase around 86% of the total purchased milk in BiH.

### Results and discussion

Based on the guidelines on good farmer practices in milk production (Scheme1.), basic problems in milk production in Bosnia and Herzegovina can be identified.

Scheme 1. The guiding objective for good dairy farming practice

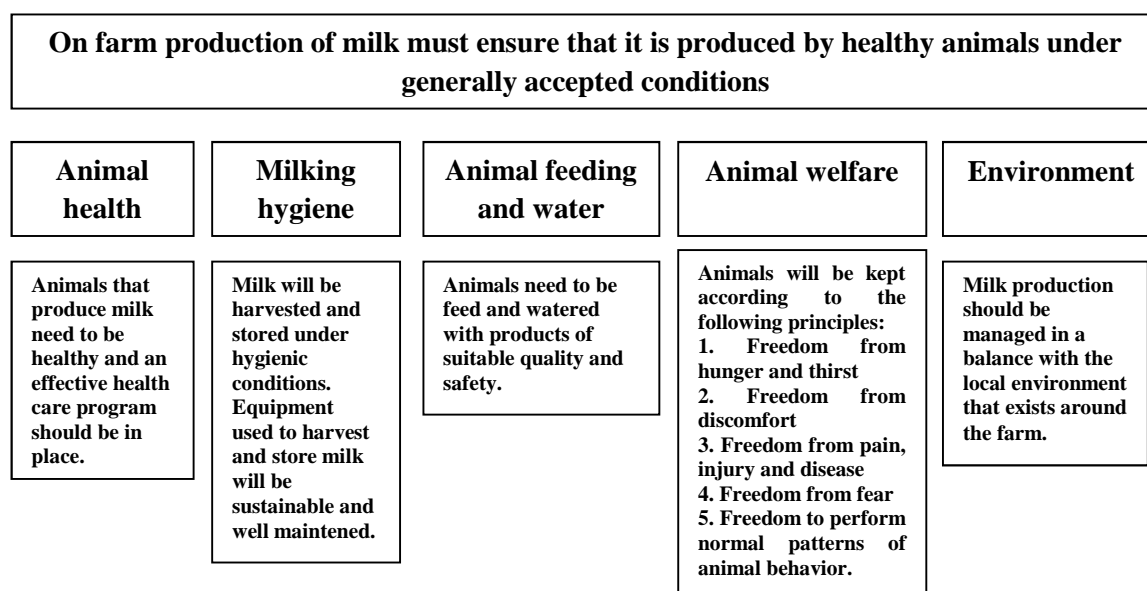


Table 1. The purchase of raw milk (litter)

Year	Federation B&H	Republic of Srpska
2010	88.306.464	75.080.400
2011	88.674.125	75.076.810
2012	100.579.225	83.876.560

(Source: Ministry of agriculture Federation BH, Republic office for statistics RS)

From table 1. is visible slightly higher purchase of milk in the Federation in relation to the purchase of milk in the Republic of Srpska.

Table 2. Number of milking cows, milk production per cow per year and total milk production

Year	Federation B&H			Republic of Srpska		
	No. cows	Milk per cow, litter	Total milk, 000 litter	No. cows	Milk per cow, litter	Total milk, 000 litter
2002	142.000	1.889	267.520	143.000	2.000	286.000
2003	153.000	1.882	278.771	131.000	2.100	275.229
2004	157.000	1.900	299.135	137.000	2.000	273.938
2005	157.000	1.947	304.895	141.000	2.232	315.029
2006	158.000	1.969	312.062	150.000	2.270	340.823
2007	150.000	2.088	313.330	145.000	2.770	401.121
2008	155.000	2.143	322.221	157.000	2.580	405.100
2009	154.000	2.163	321.096	146.000	2.800	405.183
2010	145.555	2.178	316.997	124.000	2.969	368.161
2011	140.555	2.251	315.281	120.000	2.874	344.927
2012	137.910	2.315	319.260	110.000	2.968	326.492

(Source: Federal office for statistics Federation B&H, Republic office for statistics RS)

From these table is significantly noticeable drop in the number of dairy cows since 2008. year, but it is important that the milk production per dairy cow is constantly increasing. The level of milk production per dairy cow is very low and the main factors of low milk production are shown in Scheme 2.

Slightly higher average milk production per dairy cow is in the Republic of Srpska compared to the Federation BiH.

Schema 2. Main problems in milk production in Bosnia and Herzegovina (genetic potential is OK)

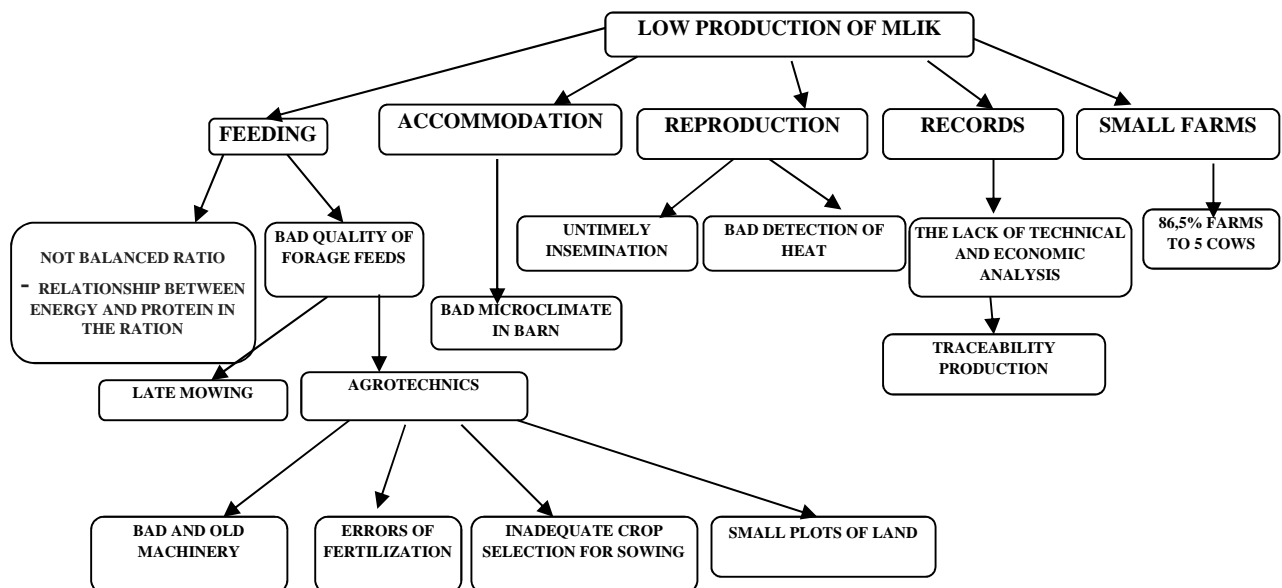




Chart 1. Information from 6 biggest dairy in BiH 2012. Year, Farm size (Source: Survey Dairy)

Based on the data obtained from our own research, the survey of six largest dairy in BiH, which purchase 86% of the total milk purchased in BiH, the data are quite different compared to the statistical data. Thus, the milk production per dairy cow is 4,081 litters and it is significantly higher than the average of the total population of dairy cows in BiH.

Chart 2. The quality class of raw milk (%) (Source: Survey laboratory)

Another important factor for the price of milk is milk quality (bacteria and somatic cells). According to data from four laboratories that are doing microbiological analysis of raw milk only 42% of the milk has extra class quality. Data for Croatia is that 93% of raw milk extra class.

A disturbing fact is the steadily increment of amount of milk that is classified outside of class, as a consequence of incorrect sample or results of the analysis that are unacceptable.

### **Conclusion**

The research results suggest that milk production in Bosnia and Herzegovina in the last few years has been changed significantly. Number of dairy cows in the 2012th is lower compared to 2002. year for about 37,000 dairy cows or about 13%, and compared to 2008. , the number of dairy cows in the 2012th year decreased by 64,090 dairy cows, or 20.50%.

The amount of milk produced in the 2012th is higher compared to 2002. year for 92.232.000 liters, or 16.6%, and the total milk production in the 2012th is lower compared to 2008. year for 61,569,000 liters or 8.47%.

Milk yield per dairy cow is on a constant rise and in 2012 in the Federation of BiH is 2,315 liters, while the Republic of Srpska is 2,968 liters per year. This milk yield is lower than the world's average for about 20%, and the European average by about 55%.

The quality of the milk produced is lagging far behind the quality of the EU and is currently only 53% of the milk in the E & I class that is allowed by Regulations on the e quality of fresh raw milk in Bosnia and Herzegovina. Due to the complexity of the task, the need for consumer protection and adaptation to EU regulations, increase of quality and health safety of milk produced in Bosnia and Herzegovina is probably one of the most challenging parts of the agrarian policy in the future.

The government support was reflected in the incentives for milk per dairy cow, for the production areas and investment support. Incentives are different at the entity level.

At the level of the state or at the entity measures should be taken to protect domestic production, as well as the adoption of legislation and regulations related to the EU standards in the production, sale and processing of milk.

1. Small dairy herds, with an average of 5 dairy cows, have no motive or preconditions for business growth. Thus, the program development of milk production in B&H should be based on three categories of producers (Glavic et al. 2012.): Modern conventional production systems adapted to small farms with an average of 15-20 dairy cows in the herd. It is estimated that these farms could make an average production of 4500-5000 liters of milk per cow per year.
2. The system of specialized family dairy farms with 40 to 50 cows in the herd. On these farms it could be made production of 5000-6000 liters of milk per cow per year.
3. System of large commercial dairy farms (with more than 100 cows in the herd), which could provide more than 6,000 liters of milk per cow per year.

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**VARIABILITY OF THE PRODUCTION CHARACTERISTICS OF THE ISOLATED TRAITS OF THE HONEY BEE IN THE AREA OF TREBINJE**

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**Abstract**

To obtain high yield in beekeeping and to exploit potential of the honeybee pasture in the region of the Republic of Srpska it is necessary to use carefully chosen honeybee queens with a good biological potential. Protection of the local subpopulations of the honeybees in order to preserve biodiversity of this kind in the Republic of Srpska is the second important factor for the choice of the honeybees mother queens and their further selection. In the paper we have examined 4 bee traits situated in the selection centre nearby Trebinje. This area is on the border of BH, Montenegro and Croatia in the zone of Mediterranean climate and it is additionally interesting for the isolation of the honeybee traits in order to have new honeybee queens in the earlier phases of beekeeping seasons than in other areas of the RS. The amount of bees, brood, honey, pollen, brood quality and behaviour was investigated in two spring and one autumn survey. All data have been carefully collected, statistically processed and analysed. Trait V2 had the biggest amount of bees in the first and second spring examination (2.26 and 4.84 frames) and statistically it is importantly different from other traits by this characteristic ( $P < 0.01$ ). The same trait had the biggest amount of brood in spring examinations and it was importantly different from the other traits ( $P < 0.01$ ). The best brood quality (mark 3) in all 3 examinations during the year had traits V2 and G2. All examined traits showed variability that provides successful improvement of wanted characteristics and selection of suitable mother queens in order to get high productivity in beekeeping.

**Keywords:** honeybee, trait, production characteristics, selection

**Introduction**

Honeybee (*Apis mellifera* L.) is the most useful insect. Apart from the direct bee products, indirect benefit of the pollination in the world is estimated at more than 150 billions euros, which makes 9,5% of the world production value. (Gallai & assiss., 2009). In beekeeping, as well as in other branches of cattle raising, a lot of attention is paid on the selection of productive and vital parentism in the process of breeding and selection. These postulates are imposed by ambition for higher yield and more economic production. This task is difficult because quantitative characteristics, such as honey yield, are affected by a few genes, and apart from it the effect of the para-genetic factors are present (Hellmich et al., 1985; Page et al., 2002). Honey yields are also defined by the number of the working bees cells and by the number of the bees in the bee hive (Szabo & Lefkovitch, 1989). In order to have a normal spring development it is necessary to have enough brood of the different age in the bee hive (Jevtic, 2007). Quick development of the brood in spring is the race characteristics of the Carniolan bee, but it can also be affected by winter food reserve (Mladenovic et al., 2002). Quantity of pollen in the bee hive is very important for the nutrition and it is the source of

proteins, fats, vitamins and minerals for bees. Between the total surface of the brood and the number of the pollen bees there is a high positive correlation (Lebedev, 2001; Mladenovic et al., 2004). Separation and selection of bee traits with production characteristics which are above the average are the aim of the selection work. Therefore, the aim of this research was to study the most important productive traits of some bee traits from surrounding of Trebinje and give recommendations for breeding of the best traits of honey bees.

### **Materials and methods**

This research was carried out on the apiary of the selection centre in Trebinje. Four bee traits of the *Apis mellifera carnica* race were included in the evaluation procedure. The first two traits V1 and V2 originates from Vucija, trait G1 from Golija and G2 from Grahovo. Traits were formed from the daughters and dominated queen mothers. The bee colonies were put into standard Langstro-Rut beehives and at the start of the trial they were equalized in terms of strength (population of bees). The evaluation was performed in two spring examinations (I and II) before the beginning of *Salvia officinalis* pasture and in the autumn examination in the first 10 days of September. In order to do so, the individual frame was though to be divided in 10 horizontal parts (5 parts per side). The numbers represent the sum of values recorded for each trait from all frames of the colony. The following productive traits were examined using the above method: the surface of comb covered with bees, brood, honey and pollen. Brood quality was recorded using a system of points: 3 = excellent (without empty cells), 2 = good (present of few individual empty cells), 1 = spotty brood. Gentleness of bees was also evaluated using the point system: 4 = keep still, 3 = restless, 2 = runs from the combs, 1 = leaves the combs.

All measurements of examined characteristics for the bees were analysed by one way ANOVA analysis and comparisons between different traits were determined by LSD Test.

### **Results and discussion**

To obtain high yield in beekeeping it is necessary to have strong colonies and one of the aims in the beekeeping technology is to have a large/numerous colony. Because of that, quantity of bees per a colony is a very important economic characteristic of honey bees which are examined in the selection work.

Results of all examined characteristics are shown in the Table 1. The biggest quantity of bees in all three examinations appeared in colonies of V2 traits from Vucija (2,26, 4,84 and 2,55 frames).

During his examination of seven traits in Timok region, Serbia in the first spring examination Georgiev (2006) defined 2.93 frames of bees per a colony, while Nedic (2009) in the first spring examination for six bee traits defined 1.46 frames of bees per a colony in average. In the first two spring examinations the difference in the quantity of bees was importantly ( $P < 0.01$ ) different between examined traits (Table 2). In LSD trait examinations Trait V2 was statistically very different from the other traits ( $P < 0.01$ ) for the surface of bees on the frame.

In order to have a good spring development in colonies it is very important to have brood in all developing phases starting from eggs, then larvae to covered brood during orchard blossoming (Jevtic, 2007). In our examinations we defined statistically very significant difference regarding the surface of brood in four examined traits in the first two spring examinations (Table 2). The biggest surface of brood in spring examination had trait V2 (2.41 and 5.29 frames) and these values are statistically very different ( $P < 0.01$ ) from the other traits.

In spring, fresh flow of nectar and pollen cannot completely satisfy colonies needs for feeding brood. Because of that bees use food stored for wintering. The lack of honey in

spring leads to the reduction of the cared larvae and it reduces the colony strength for the main pasture. (Taranov, 2001).

The quantity of honey among examined bee traits is significantly different in the first spring examination, while in the second spring examination and in autumn examination there was not statistical difference in the amount of honey among traits. For this characteristic also Trait V2 had the biggest average amount of honey (1.16 and 3.64 frames) in the first two spring examinations.

Table 1. Descriptive statistics for the examined traits of honeybee traits

Factor	I Spring exam			Factor	II Spring exam			Factor	Autumn exam		
Trait	n	$\bar{x}$	SD	Trait	n	$\bar{x}$	SD	Trait	n	$\bar{x}$	SD
Honeybee surface (1/10 frame)											
V1	8	1,09	0,39	V1	8	2,88	0,66	V1	8	1,73	0,55
V2	8	2,26	1,05	V2	8	4,84	1,13	V2	8	2,55	0,95
G1	4	0,88	0,30	G1	4	2,58	1,16	G1	4	1,60	0,58
G2	6	1,15	0,59	G2	6	2,83	2,08	G2	6	2,02	0,67
Average	26	1,43	0,87		26	3,42	1,56		26	2,03	0,78
Brood surface (1/10 frame)											
V1	8	0,99	0,27	V1	8	3,55	0,77	V1	8	3,43	0,57
V2	8	2,41	1,21	V2	8	5,29	0,96	V2	8	3,70	1,36
G1	4	0,83	0,51	G1	4	3,43	1,46	G1	4	3,45	0,84
G2	6	1,15	0,58	G2	6	2,65	1,54	G2	6	3,97	0,72
Average	26	1,44	0,99		26	3,86	1,49		26	3,64	0,92
Honey surface (1/10 frame)											
V1	8	1,03	0,37	V1	8	1,30	0,34	V1	8	0,99	0,35
V2	8	1,16	0,46	V2	8	3,64	2,74	V2	8	2,94	2,15
G1	4	0,80	0,35	G1	4	1,80	1,33	G1	4	1,43	1,72
G2	6	0,57	0,20	G2	6	1,92	2,11	G2	6	2,75	1,25
Average	26	0,93	0,42		26	2,24	2,05		26	2,06	1,67
Pollen surface (1/10 frame)											
V1	1	0,10	0,00	V1	8	0,28	0,15	V1	8	0,76	0,38
V2	4	0,13	0,05	V2	8	0,78	0,58	V2	8	0,78	0,31
G1	0	-	-	G1	2	0,60	0,71	G1	3	0,23	0,06
G2	1	0,30	0,00	G2	5	0,48	0,50	G2	5	0,52	0,24
Average	6	0,15	0,08		23	0,52	0,48		24	0,65	0,35
Brood quality (mark from 3 to 1)											
V1	8	2,75	0,46	V1	8	3,00	0,00	V1	8	3,00	0,00
V2	8	3,00	0,00	V2	8	3,00	0,00	V2	8	3,00	0,00
G1	4	2,75	0,50	G1	4	3,00	0,00	G1	4	3,00	0,00
G2	5	3,00	0,00	G2	6	3,00	0,00	G2	6	3,00	0,00
Average	25	2,88	0,33		26	3,00	0,00		26	3,00	0,00
Gentleness (mark from 4 to 1)											
V1	8	4,00	0,00	V1	8	4,00	0,00	V1	8	3,50	0,53
V2	8	3,88	0,35	V2	8	4,00	0,00	V2	8	3,63	0,52
G1	4	4,00	0,00	G1	4	4,00	0,00	G1	4	3,75	0,50
G2	5	3,80	0,45	G2	6	4,00	0,00	G2	6	3,67	0,52
Average	25	3,92	0,28		26	4,00	0,00		26	3,62	0,50

To satisfy feeding needs of honey bees in proteins it is necessary to have pollen of the adequate quality and quantity in bee colonies (Mladenovic et al., 1999). In the examined traits we noticed the lack of pollen in the first spring examination, where pollen was found only in six colonies. This lack bees try to overcome in spring, what is seen in the second spring examination. Low values of the marked pollen in all traits in the second spring examination are the result of the high consumption of pollen in the nutrition of the bee brood. Brood quality is the characteristic of high importance during selection work. Loose and spotty brood can be a sign of mating in relationship, or it can also be a sign of an old bee queen or not quality bee queen. Based on the results of examination of brood quality it is established that only traits V2 and G2 had brood marked with best marks (3.00), while traits V1 and G1 in the first spring examination had brood of less quality which was marked with 2.75.

Table 2. Results of analysis of one way ANOVA for examined traits

Factor	I Spring exam		II Spring exam		Autumn exam	
	F <sub>exp.</sub>	p	F <sub>exp.</sub>	p	F <sub>exp.</sub>	p
	Honeybee surface					
Traits	5,52	0,01**	4,54	0,01**	2,294	0,106
	Brood surface					
Traits	6,23	0,00**	6,75	0,00**	0,436	0,730
	Honey surface					
Traits	3,35	0,04*	2,18	0,12	2,906	0,057
	Pollen surface					
Traits	5,50	0,10	1,59	0,22	2,905	0,060
	Brood quality					
Traits	1,21	0,33	-	-	-	-
	Gentleness					
Traits	0,69	0,57	-	-	0,241	0,867

\* -  $P < 0,05$ ; \*\* -  $P < 0,01$ ;

Behaviour of the Carniolan bees is very nice and it is known as a tranquil bee which does not show aggression. During selection work the aim is to choose bees that area tranquil and that do not sting during examinations of colonies. This characteristic can be influenced not only by genetis predisposition, but also by environment temperature, different smells that can irritate bees etc. Because of that it is important to have the same criteria for the whole apiary where are colonies are situated from traits. The average behaviour mark for all examined traits was the best during the second spring examination (4.00), while in the first spring and autumn examination there were differences in behaviour of examined traits that influenced the average behaviour mark (3.92 and 3.62). The best behaviour mark was given to the trait G1 (4.00, 4.00 and 3.75), and the worst behaviour mark was given to the trait V2 (3.88, 4.00 and 3.63). Defined differences between examined traits were not statistically important ( $P > 0.01$ ). The similar results were also got by Georgieva (2006) while examining bees from the area of East Serbia and she marked behaviour in her selection examinations with 3.47, 3.76 and 3.78.

## Conclusion

Based on the results of the examined productive characteristics: bee surface, brood surface, honey surface, pollen surface, brood quality and bee behaviour in four honey bee traits in the selection centre in Trebinje we can make the following conclusions:

Examined traits showed the characteristic productive characteristics for the Carniolan bee race. The best results were found in trait V2 (Vucija 2) especially for the characteristics of bee surface and brood in the first two spring examinations and statistically very significant ( $P < 0.01$ ) it differed from the rest of examined traits. Trait V2 had also a good honey surface during examinations and an excellent mark for brood quality.

Only behaviour of trait V2 was worse comparing to the other traits, but this characteristic can be improved in the selection work by choosing mothers with tranquil temperament. Results of trait V2 can be explained by the fact that locally adapted organisms, such as honey bees, have better productive results than the organisms bred in other ecological conditions.

Shown variability of the examined characteristics gives enough space to improve the most important productive characteristics by careful selection of bee queens mothers from traits with results that are above average. By extension of the high-quality breeding mothers we can expect positive effects of the application of selection in the beekeeping in the Republic of Srpska.

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## EFFECT OF BODY WEIGHT HENS ON REPRODUCTIVE TRAITS OF BROILER BREEDERS

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### Abstract

Investigation of the effect of body weight on reproductive performance of hens of broiler breeders was conducted in the hybrid Ross 308 and Cobb 500. At the beginning of the production cycle (24 weeks old) with hybrids Ross 308 determined the average body weight of hens 2680.40 g and 2697.80 g Cobb 500. After 42 weeks of age (mid-production cycle) weight hens was 3565.10 g (Ross 308) and 3599.05 g (Cobb 500), while at the end of the production cycle (61 weeks old) weight layers in the hybrid Ross 308 was 3841.50 g, and Cobb 500 3850.00 g. The differences found in body weight of laying hens (17:40 g, 33.95 g 8:50 g) in certain periods of the production cycle, and the difference in body weight of hens for full production cycle (23:26 g) were not statistically significant ( $P > 0.05$ ). Specifically assess the influence of body weight on reproductive performance of hens of broiler breeders was determined by calculating the coefficient of phenotypic correlation between the studied parameters. Thus, the association between body weight and most hens reproductive indices of broiler breeders was positive statistically significant ( $P < 0.001$ ) correlation coefficient of phenotypic correlation, while the association between body weight and percentage hen chicken hatching of fertilized eggs defined negative correlation coefficients that were not statistically significant ( $P > 0.05$ ), and between the layers of body weight and relative weight loss of eggs, but statistically significant ( $P < 0.001$ ).

**Key words:** hens, body weight, reproductive traits, broiler parents.

### Introduction

In addition to the optimal sex ratio and age of broiler breeders on fertility and egg hatching and has a significant influence body weight, as hens, and roosters during the period of egg production (Savic et al., 2004; Ciacciariallo and Gous, 2005; Djermanovic et al. 2005; Vieira et al., 2005; Almeida et al., 2006; Djermanovic et al., 2008; Milosevic and Peric, 2008; Djermanovic et al., 2009; Djermanovic, 2010; Djermanovic et al., 2010; Mitrovic et al., 2010; Djermanovic et al., 2013). The proper functioning of the endocrine system and hormonal hens besides age and photostimulation (Lewis et al., 2005; Lewis and Gous, 2006, 2007; Usturoi et al., 2007) to a large extent depends on body development of breeding birds. At optimum body weight at a given age in the layers stimulates the ovary, and thus accelerates the maturation of oocytes, or egg production.

To the fertilized egg brought offspring, it is necessary that the fertilized egg cell, provide the necessary conditions for the development of the embryo. Only proper diet (Wilson, 1991; Barnett et al., 2004; Maiorka et al., 2004; Enting et al., 2007; Wolanski et al., 2007), breeding technology generation and utilization of parent stock can be provided pre-condition for the maximum percentage of the study and the necessary vitality and quality of eggs for incubation and izleženog offspring (Luquetti et al., 2004; Hesn Sahin et al., 2009; Schmidt et al., 2009). To the period of production of fertile eggs or day-old chicks, lasted longer period



is necessary to keep hens kept in priplodnoj shape, with special attention to the weight. It should also be borne in mind that uniformity in terms of weight especially significant factor in the second half of the production cycle.

Body weight of breeding birds, in addition to other factors, especially age, has a direct impact on reproductive performance. Therefore, special attention is paid to the influence of body weight hens during the production cycle on reproductive performance of broiler breeders. The main objective of this study was to determine the kind of impact a body weight of hens intensity weight egg hatching of fertilized eggs, day-old chicks weight, the relative proportion of chicken in egg weight, egg weight loss absolute and relative weight loss of eggs.

### **Material and Methods**

The investigations included two parent flocks of broilers chickens Ross 308 and Cobb 500 During the production cycle used the technology proposed by the breeders of the respective hybrids ([www.rossbreeders.com](http://www.rossbreeders.com); [www.cobb-vantress.com](http://www.cobb-vantress.com)). Both broiler parent flocks were kept on the floor with deep litter and feeding, watering, ventilation and lighting are automatically regulated. Effective floor area per building was about 900 m<sup>2</sup>, with a population density in the exploitation of both studied broiler parent flocks was about 6 birds/m<sup>2</sup> floor area.

Broiler breeder flocks tested were grown to 61 weeks of age, ie. both clusters spread the early 22 week, and were used for the incubation of eggs laid 24 weeks of age until the end of the production cycle, because then met the minimum weight of a suitable incubation (> 50.00 g). From the foregoing it follows that the period of production of eggs and day-old broiler production lasted for 38 weeks (from 24 to 61 weeks old broiler breeder).

As an initial experimental material has served a total of 5200 birds of both sexes Ross hybrids 308 and 5430 broiler breeder Cobb 500, grown in two separate building. The first building was moved in 4750 and 450 hybrid Ross 308, and in 4960 another and 470 Cobb 500, so that the sex ratio are 1: 10:56 (Ross 308) and 1:10:55 (Cobb 500).

In the preparatory period of 21 to 24 weeks of age mortality and culling flocks of laying hens in the hybrid Ross 308 was 13 birds (0.279%), and in 12 of Cobb 500 birds (12:24%). This means that at the beginning of the use of eggs for incubation in hybrids Ross broiler breeder flock was 308 in 4737 hens, or hens, 4948 Cobb 500.

In order to control body weight each week individually measured 400 hens selected randomly, ie. per 200 hens hybrid Ross 308 and Cobb 500 These measurements followed the uniformity laying flocks tested during the production cycle, and then examined the influence of body weight on laying the basic reproductive performance of broiler breeder (egg weight, hatching of fertilized eggs, day-old chicks weight, the relative proportion of chicken in egg weight, absolute weight loss eggs and egg weight relative loss).

Primary data processing was performed by standard variational - statistical methods, and testing the differences between the hybrids using the t-test. For all monitored parameters are calculated average values, error of the mean and standard deviation. In addition, the obtained results were used to calculate association and dependence of the traits by week production using correlation analysis. Statistical analysis was performed using Analyst software program SAS / STAT (SAS Institute, 2000).

### **Results and Discussion**

Average, variability and significant differences in body weight in laying hens at certain periods of the production cycle, or for the whole period of egg production are shown in Table 1.

**Table 1** Average, variability and significant differences in body weight hens (g) of the period of production cycle

The period of production cycle	Weeks of age (production)	Hybrid	$\bar{x} \pm \text{SEM}$	S	$\bar{d}$
Start	24 (1)	Ross 308	2680.40±14.63	206.93	17.40 <sup>ns</sup>
		Cobb 500	2697.80±17.09	241.66	
Middle	42 (19)	Ross 308	3565.10±19.86	280.92	33.95 <sup>ns</sup>
		Cobb 500	3599.05±20.12	275.28	
End	61 (38)	Ross 308	3841.50±21.39	302.56	8.50 <sup>ns</sup>
		Cobb 500	3850.00±21.68	306.59	
Whole production cycle	61 (38)	Ross 308	3411.15±61.58	394.33	23.26 <sup>ns</sup>
		Cobb 500	3434.41±61.03	390.76	

<sup>ns</sup>P>0.05.

Data Table 1 shows that the average body weight of hens of each strain increased gradually during the production cycle. Body weight of hens in 24 week was 2680.40 g (Ross 308) and 2697.80 g (Cobb 500), and at the end of exploitation (61 weeks old) 3841.50 g in the hybrid Ross 308 and 3850.00 g in Cobb 500. During the production cycle of Cobb 500 hens compared to hens hybrid Ross 308 had a higher average body weight, which was not statistically confirmed (P> 0,05). The average body weight of hens hybrid Ross 308 for the entire period of exploitation was 3411.15 g, and Cobb 500 3434.41 g, where the difference in body weight of laying hens (23:26 d) between the hybrids was not statistically significant (P> 0.05), indicating that genotype had no significant effect on body weight of laying hens.

Body weight of hens of each strain, was slightly higher than expected genetic potential. Similar results, in terms of average body weight of hens came Djermanovic (2010), Djermanovic et al. (2009) and Mitrovic et al. (2010). Usturoi et al. (2007) have each control measurement during rearing of broiler breeders Ross 308 hybrid found a slightly lower average body weight of hens, which according to the groups of hens, 60 weeks of age was between 3988.95 g 3990.44 g. Lewis et al. (2005) and Lewis and Gous (2006) in 60 week old Cobb 500 hens found a significantly higher average body weight of hens, between 4:21 and 4:25 kg. Also, a significantly higher average body weight of hens hybrid Ross 308 (4.43 kg) and Cobb 500 (4:56 kg) 59 weeks of age they found Lewis and Gous (2007).

Production of day-old broiler chickens of the final phase of the breeding parent flocks severe type. Therefore, apart from the flock age and body weight hens and roosters, and special attention should be paid to a number of indicators (Table 2) which determine the success of the respective type of production.

**Table 2** Average values and variability of difference reproductive indices of broiler breeders

Indicators	Hybrid	$\bar{x} \pm \text{SEM}$	S	$\bar{d}$
Egg weight, g	Ross 308	62.03±0.80	4.96	0.22 <sup>ns</sup>
	Cobb 500	62.25±0.90	5.57	
Hatching of fertilized eggs,%	Ross 308	82.79±1.28	7.87	0.89 <sup>ns</sup>
	Cobb 500	83.68±1.39	8.60	
Weight day old chicks, g	Ross 308	39.85±0.70	4.34	0.17 <sup>ns</sup>
	Cobb 500	40.02±0.78	4.79	
The relative proportion of chicken in egg weight,%	Ross 308	64.05±0.37	2.26	0.07 <sup>ns</sup>
	Cobb 500	64.12±0.38	2.32	
The absolute loss of egg weight, g	Ross 308	22.19±0.19	1.15	0.05 <sup>ns</sup>
	Cobb 500	22.24±0.20	1.26	
Relative egg weight loss,%	Ross 308	35.88±0.37	2.26	0.00 <sup>ns</sup>
	Cobb 500	35.88±0.38	2.32	

<sup>ns</sup>P>0.05.

Similarly, the values of average body weight layers (Table 1) between the studied reproductive parameters of broiler breeders were also not statistically significant (P> 0.05) differences (Table 2).

Regardless of genotype and other authors who have studied this issue have found that the age of the flock, and therefore increased body weight broiler breeder hens of different genotypes increased egg weight. Thus, Wilson (1991) and Lewis and Gous (2007) found a similar average weight of eggs, eggs greater weight determined Luquetti et al. (2004) and Vieira et al. (2005), significantly higher Almeida et al. (2006), while Wilson (1991) and Maiorka et al. (2004) found a slightly smaller, and much less Barnett et al. (2004) and Ciacciarriello and Gous (2005). Similar results on the feasibility of fertilized eggs from the chickens came Savic et al. (2004), Djermanovic et al. (2005), Djermanovic et al. (2008) and Djermanovic (2010) at approximately the same period of exploitation (36, 44 and 38 Sunday) of broiler breeders, while slightly worse results found Mitrovic et al. (2009), and much worse Milosevic and Peric (2008).

To the conclusion that the burden of day old chicks, similar to egg weight, increases with age and weight gain hens came Luquetti et al. (2004), Maiorka et al. (2004), Barnett et al. (2004), Vieira et al. (2005), Almeida et al. (2006), Wolanski et al. (2007), Enting et al. (2007), How to become Sahin et al. (2009) and Djermanovic (2010). Significantly higher average weight of day old chicks hybrids Ross determined Vieira et al. (2005) and Almeida et al. (2006) with Cobb. According to the assessed valuation of egg weight and chick weight to similar results in terms of relative share of chicken in egg weight came Luquetti et al. (2004), Maiorka et al. (2004) and Djermanovic (2010), while higher values of the above parameters determine Barnett et al. (2004), How to become Sahin et al. (2009) and Schmidt et al. (2009).

In addition to established measures of variation for body weight and reproductive indices of laying hens analyzed parent flocks, in order to better analyze the impact of body weight on reproductive performance of laying hens, calculate the correlation coefficient of phenotypic correlation between the traits (Table 3).

**Table 3** Phenotype Correlation of body weight hens and broiler breeder reproductive indices

Hibrid	Indicators	Correlation coef. ( $r_p$ )
Ross 308	Body weight of hens (g): Weight of eggs (g)	0.989 <sup>***</sup>
Cobb 500		0.989 <sup>***</sup>
Ross 308	Body weight of hens (g): hatching of fertilized eggs (%)	-0.126 <sup>ns</sup>
Cobb 500		-0.097 <sup>ns</sup>
Ross 308	Body weight of hens (g): Weight day old chicks (g)	0.962 <sup>***</sup>
Cobb 500		0.960 <sup>***</sup>
Ross 308	Body weight of hens (g): The relative contributions of chicken in egg weight (%)	0.768 <sup>***</sup>
Cobb 500		0.765 <sup>***</sup>
Ross 308	Body weight of hens (g): Absolute weight loss of eggs (g)	0.644 <sup>***</sup>
Cobb 500		0.719 <sup>***</sup>
Ross 308	Body weight of hens (g): Relative egg weight loss (%)	-0.768 <sup>***</sup>
Cobb 500		-0.765 <sup>***</sup>

<sup>ns</sup>P>0.05; <sup>\*\*\*</sup>P<0.001.

Data Table 3 show that between body weight hens and egg weight, body weight and weight of hens day old chicks define a complete correlation connection in both studied hybrids. Also, the body weight between hens and most reproductive parameters of broiler breeders phenotypic correlation coefficients of correlation were positive statistically significant (P <0.001). Between body weight and percentage hen chicken hatching of fertilized eggs were established negative correlation coefficients that were not statistically significant (P > 0.05), and between the layers of body weight and relative weight loss of eggs but statistically highly significant (P <0.001).

A similar, but opposite results in terms of connectivity and dependency productive and reproductive performance of different genotypes of broiler breeders came Djermanovic et al. (2005), Djermanovic et al. (2008), Mitrovic et al. (2009), Djermanovic (2010), Djermanovic et al. (2010) and Mitrovic et al. (2010).

### Conclusion

Based on the obtained results it can be concluded that the average body weight of laying hens in both studied hybrids in relation to engineering standard, smaller, both at the beginning and at the end of the production cycle. However, the difference between the weight layers of the hybrids were not statistically significant ( $P > 0.05$ ), and genotype had no significant effect on body weight of laying hens.

The calculated coefficients of phenotypic correlations and their significance, we can say that the hens body weight significantly affected their breeding ability because of both parent flocks among the traits, identified statistically significant ( $P < 0.001$ ) correlation coefficients, except between body weight hens and chicken hatching percentage of fertilized eggs ( $P > 0.05$ ), this suggests that the increase in body weight decreases the ability of breeding hens, which causes considerable shortening of the production process.

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## THE PELLETING PROCESS OF DIFFERENT MATERIALS AND IMPACT ON ENERGY CONSUMPTION

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### Abstract

The paper presents effects of moisture methods of different raw materials process and influence on energy compounds in pelleting process. There are many benefits to pelleting of food. Improving feed conversation in animal, easier handing of feed and storage, balanced diet of animal, less wastage of food and many other benefits. The experimental materials involved maize wheat and barley. The raw materials was ground throught a 3 mm hammer mill. Condition materials was with cold procedure. Moisture of materials were 14, 16, 18, 20, and 22%. Moisture is important step in hight quality pellet production. The experimental materials (maize, wheat and barley) were brought to temperature of 70 ° C. The pelleting process were done in Metalac-Ostojic, pellettizing produced in Obrenovac – Srbija capacity 2-2.5 t/h. Important consideration in feed industry is per tone of pellet production. One factor is to reduce input such is energy consumption. In peper is present different moisture and getting appropriate parameters can influence on the amount of power consumption in maze, wheat and barley. The test materials (maize, wheat and barley) before milling processes humidity was 13 percent.

The relationship between parameters of moisture and energy consumption in this process analyzed statistical procedures analysis of Varansa (ANOVA).

One of the primary objectives of all commercial feed manufactures is to economically produce the best pellet quality possible. There are numerous factor that affect pellet quality, cost pellet and many are inter-related.

**Key words:** energy, pelleting, moisture

### Introduction

The pelleting process is the most expensive and complicated process in feedmill, however the benefits of pellets include: decreased feed wastage, reduced selective feeding, distribution. However pelleting increasing the cost of feeds because cost pelletires is relativety high compared to grinders or mixer. The energy requirements is high and additional care and skill is necessary for that operation. The Scoch ( 1981. ) found that steam conditioning led to decrease is mechanical fruction during pelleting, as determined by low temperature across the pellet decreased electric energy consumption. Jean-Jacques (2005.) show a variation power consumption in fodder factories.

Pellets average 74 kwh/t minimum 59,5 kwh/t and maximum 101,8 kwh/t consumption. Properly conditioned with good durability , hardness and hygienie quality together with improved nutritional value of feed (Thomas, 1997).

Water and steam addition reduced energy cconsumption of pellet press (Djuro Vukomirovic, 2010.) in process pelleting moisture of material must be in optimum between 15-18%. (Slavica Sredanovic, 2000.)

Conditing is one of the key unit operation in pellet production. High moisture contains conditioned material resulted in decreasing of energy consumption during pelleting process (Djuro Vukomirovic, 2010.)

Heat treatment during the pelleting process reduces microorganisms. The overall hygiene state is improved storage periods may be extended – up to storage conditions decay is deferred. (Raine Lowe, 2010.)

Amount of time it takes to produce a given quantity of feed and calculate t/h . Then apply the following formula to calculate power (Kw) being take by pellet press motor.

$$Kw = \text{average pellet press} \times \text{motor amperage} \times \text{voltage} \times 1,73 \times \text{power factor} / 1000$$

Power factor 0,93.

### Materials and methods

Examination mill is done on agricultural firms in Kaonik-Srbija in Ekomlek in the unit Ekofarm. The pelleting process was done in Metalac-Ostojic pelletizing produced in Obrenovac capacity 2,5 t/h. The experimental materials were maize (*zea mays*), wheat (*triticum aestivum*) and barley (*hordeum vulgare*).

In this study samples were taken moisture of materials 14, 16, 18, 20, and 22%. The test material (maize, wheat, and barley) before milling process were 13% moisture.

The used temperatures in pelleting were 70-80%. The raw materials was ground throught 3 mm hammer mill.

The analysis of dependen between the parameters of pelleting process was carried using statistical procedures (ANOVA).

### Results and disscussion

Conditie is an important step in high quality pellet production. The optimum temperatures in pelleting process is 70-80%. Higher temperature affect the chemical structure of pelleting materials.

Table 1.Average chemical composition of the tested materials

<i>Grains</i>	<i>Carbon hydrates</i>		<i>Cellulase</i>	<i>Fat</i>	<i>Protein</i>
	<i>Starch</i>	<i>Remaining</i>			
<i>Wheat</i>	64,08	6,30	2,5	1,75	12,35
<i>Maize</i>	62,57	8,33	2,1	4,62	9,85
<i>Barley</i>	61,76	7,60	5,4	2,16	11,14

In table 1 as can be seen that tested materials barley has most celluloses (5,4%) and corn most fat (4,62%) it impact to pelleting process.

Examining the impact of individual grains on the energy consumption during pelleting, may influence the composition of complete diets in animal nutrition and therefore the price of production.

Energy consumption during pelleting separate studies in each case. Raw material moisture was 14%. Final moisture contains 14%, 16%, 18%, 20%, and 22%.

Table 2. Dependence of the energy consumption (Ec-kWh/t) during pelleting of different raw materials (wheat, maize, barley)

Energy consumption (Ec-kWh/t)			
Moisture %	Wheat	Maize	Barley
14	43,90	43,00	44,50
16	43,00	42,40	43,60
18	41,90	41,00	42,60
20	41,80	40,80	42,80
22	41,80	40,80	42,80
X	42,48	41,60	43,10
—	0,942	1,03	0,97
X ave-	42,393		

	<i>df</i>	<i>ss</i>	<i>ms</i>	<i>F</i>	<i>P-value</i>
<i>Source treatments</i>	2	5,681	2,841	2,9519	0,0907
<i>Error</i>	12	11,548	0,962		
<i>Total</i>	14	17,229			

The results between mixture of raw materials (wheat, maize, barley) and consumption electrical energy in pelleting process show in table 2.

The results point to a very strong linear dependency between analysis parameters.

The smallest energy consumption was observed for maize and highest in barley

Figure 1. Relationship between moisture of raw materials and consumption electrical energy during

Results of the study investigation, the effect of moisture different raw materials show in figure 1.

**Conclusion**

Moisture condition is very important in pelleting process choosing of properly conditioning parameters and raw materials provide saving of electrical energy consumption. The optimum condition for raw materials (wheat, maize, barley) were 16-18%.

Properties of raw materials and moisture in pelleting process is a good predictor of energy consumption of the pelleting process, it is a very strong dependency between those parameters. The higher humidity of material is not significant for energy consumption.

The investigation published in this paper is part of the improvement of biotechnological processes in the function of the rational use of energy, increase productivity



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## NON-GENETIC FACTORS AFFECTING BIRTH WEIGHT OF GOAT KIDS

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### Abstract

The aim of the study was to determine paragenetic factors and effect of environmental (year) on body mass of goat kids at birth.

The goats included in this research were representatives of three breed groups – group A (Bulgarian White Dairy goat), group B (Bulgarian White Dairy goat x Toggenburg) and group C (Bulgarian White Dairy x Anglo-Nubian).

Average body mass at birth of male kids was heavier than that of females and the difference is not statistically significant ( $P > 0.05$ ). Exceptions are observed only in 2010 at Bulgarian White Dairy goat, where females are heavier than male kids –  $3.66 \pm 0.29$  to  $3.40 \pm 0.17$ , as this is probably due to the age of the goats.

The singles were heavier than twins and triplet kids and the differences are statistically significant ( $P < 0.05$ ). For the three groups average trend in singles, twins and triplets, is respectively: 2009 –  $3.25 \pm 0.149$  and  $3.12 \pm 0.15$ ; 2010 –  $3.60 \pm 0.20$ ,  $3.55 \pm 0.09$  and  $3.26 \pm 0.13$ ; 2011 –  $3.68 \pm 0.19$ ;  $3.46 \pm 0.07$  and  $3.33 \pm 0.18$ ; 2012 –  $3.97 \pm 0.14$ ;  $3.70 \pm 0.09$  and  $3.14 \pm 0.28$ .

**Key words:** goat, kids, sex of kids, birth weight, non-genetic factors

### Introduction

Birth weight is an important economic indicator for animal production. There is a positive correlation between birth weight and further increasing of the live weight of animals. (Roy et al. 1989).

Studies of various authors show that birth weight is influenced by gender, type of birth, breed, nutrition, year, season of birth, maternal age and more. (Shetaewi et al, 2001; Kumar et al., 2007; Supakorn and Pralomkarn, 2009; Bharathidhasan et al, 2009).

The results can be used to evaluate the effectiveness of selection on birth weight indicator in goat population, the impact of non genetic factors to be taken into account in breeding programs. (Montaldo et al. 1987).

The aim of the study was to determine the effect of some non-genetic factors on the birth weight of goat kids from three breed groups.

### Material and methods

The study was conducted in the experimental farm of the Institute of Mountain Stockbreeding and Agriculture (Troyan) in four year period (2009 – 2012 ). The site is located at an altitude of 300-499 m, latitude 42.883N degrees and longitude 24.717E.

The goats were of three breed groups – group A (Bulgarian White Dairy goat), group B (Bulgarian White Dairy goat x Toggenburg) and group C (Bulgarian White Dairy x Anglo-Nubian). The goat kids were born during the months of February-March.

In winter the animals were kept indoor and daily ration consisting of 1 kg/head hay, 2 kg silage and 0.4 kg concentrate. Water and salt were offered ad libitum. During the summer months (May-August) the goats were grazing.

After birth the following indicators of goat kids were reported: birth weight, sex, type of birth, year, breed and age of mother. The influences of year and maternal age on birth weight of kids are discussed in our other article.

The results of the average weight of kids at birth are viewed in various aspects: by years for each breed group and years average for the three groups and by gender and type of birth.

The data are presented as the arithmetic mean ( $\bar{x}$ ) and average standard Error ( $S\bar{x}$ ). To determine significance ANOVA Single Factor is used.

## Results and discussion

### 1. Influence of sex of kid on the birth weight.

The data for average birth weight of male and female kids through four years of study (2009 – 2012) are shown in Table 1.

Table 1. Average weight (kg) at birth of male and female kids for the period 2009 – 2012

Group	Female kids, kg.		Male kids, kg.	
	number	$\bar{x} \pm S\bar{x}$	number	$\bar{x} \pm S\bar{x}$
2009				
Group	4	2.58±0.42	5	3.30±0.49
Group B	5	2.82±0.26	4	3.78±0.18
Group	3	3.03±0.15	4	3.38±0.13
Average for , B and	12	2.79±0.17	13	3.47±0.19
2010				
Group	5	3.66±0.29	5	3.40±0.17
Group B	6	3.38±0.14	12	3.53±0.10
Group	5	3.16±0.25	3	3.57±0.23
Average for , B and	16	3.40±0.16	20	3.50±0.10
2011				
Group	13	3.28±0.11	7	3.57±0.20
Group B	5	3.26±0.14	12	3.61±0.14
Group	5	3.38±0.19	6	3.77±0.18
Average for , B and	23	3.30±0.08	25	3.64±0.09
2012				
Group	15	3.54±0.19	27	3.66±0.17
Group B	7	3.84±0.25	15	3.99±0.18
Group	12	3.83±0.17	8	4.14±0.23
Average for , B and	34	3.70±0.12	50	3.83±0.11

The data show that the average live weight at birth of male kids in all three groups is higher than that of females, during the four years of study and the differences are insignificant ( $P > 0.05$ ). Exceptions are observed only in 2010 at Bulgarian White Dairy goat, where females are heavier than male kids –  $3.66 \pm 0.29$  to  $3.40 \pm 0.17$ , as this is probably due to the age of the goats. We believe that the insignificance of this indicator is due to greater variation in birth weight in the group of kids in the male group compared to the females.

Elabid (2008) reported for higher birth weight of male than that of female kids in Sudanese Nubian goats which obtain an average live weight of male kids  $2.374 \pm 0.622$  kg and in females –  $2.312 \pm 0.481$  kg where the difference is significant ( $P < 0.05$ ).

Castillo et al. (1976) have also received significantly higher live weight of male kids at birth compared to females in breeds Anglo-Nubian, Toggenburg and Saan goat. According to Hafez (1962) higher live weight of males is due to the anabolic effect of male hormones. In table 1 is shown that the female and male kids-crosses between Bulgarian White Dairy goat and Anglo-Nubian have the highest birth weight, which we believe is due to breed.

## 2. Influence of type of birth on birth weight

Table 2 shows the variation of live weight at birth, according to the type of birth of the kids for the same period.

Table 2. Average live weight (kg) at birth according to type of birth for the period 2009 – 2012.

Group	Singles		Twins		Triplets	
	number	average, kg	number	average, kg	number	average, kg
2009						
Group			9	2.98		
Group B	3	3.33	6	3.20		
Group	1	3.00	6	3.27		
Average for , B and	4	3.25	21	3.12		
2010						
Group	1	3.40	6	3.63	3	3.37
Group B			12	3.51	6	3.42
Group	1	3.80	4	3.55	3	2.83
Average for , B and	2	3.60	22	3.55	12	3.26
2011						
Group	2	3.50	18	3.37		
Group B	2	3.90	12	3.48	3	3.33
Group	1	3.60	10	3.59		
Average for , B and	5	3.68	40	3.46	3	3.33
2012						
Group	16	3.83	22	3.54	5	3.14
Group B	10	4.09	14	3.89		
Group	12	4.04	8	3.81		
Average for , B and	38	3.97	44	3.70	5	3.14

Our results show that the type of birth has significant effect ( $P < 0.05$ ) on birth weight of kids and with increasing the number of kids born of a goat reduces their birth weight.

For the three groups average trend in singles, twins and triplets, is respectively: 2009 –  $3.25 \pm 0.049$  and  $3.12 \pm 0.15$ ; 2010 –  $3.60 \pm 0.20$ ,  $3.55 \pm 0.09$  and  $3.26 \pm 0.13$ ; 2011 –  $3.68 \pm 0.19$ ;  $3.46 \pm 0.07$  and  $3.33 \pm 0.18$ ; 2012 –  $3.97 \pm 0.14$ ;  $3.70 \pm 0.09$  and  $3.14 \pm 0.28$ .

In the study carried out in goats breed Sirohi, Banerjee and Jana (2010) reported that singles have higher birth weight than twins and they have higher than triplets. This is in agreement with our results.

Several authors (Tuah et al., 2002; Afzal et al., 2004) – in Beetal goats; Thiruvankadan et al., (2008) – in goats Tellicherry determined significant influence of the birth type on the birth weight in goat kids.

Heavier birth weight in singles kids might be attributed to uterine environment which the fetus does not have to share with its littermates, thereby attaining higher bodyweight than the twin or triplet born kids (Banerjee and Jana, 2010).

### Conclusions

1. The study shows that the average live weight at birth of male kids was higher than that of females in all three groups during the four years of the experiment.
2. It was found that on average birth weight of single was higher than that of the twins which in turn were heavier than the triplets.

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**INFLUENCE OF SEX AND LITTER SIZE OF BIRTH WEIGHT AND INCREASE  
LAMBS SJENICA IMPROVED TUFT SHEEP IN INTENSIVE BREEDIN SYSTEM**

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**Abstract**

In the two years on the family farm in the vicinity of Visoko, were monitored parameters in 35 animals Sjenicka pramenka. The sheep were kept in intensive farming systems. During the year the stock has been held for at least 10 months in a barn (in stable breeding). Annually, a minimum of seven to eight months of basic diet of sheep is corn silage. The aim of this study was to determine the influence of litter size and sex on birth weight and weight gains of lambs to weaning (90 days). The average birth weight of male singles was 5,61 kg and was significantly ( $P<0,05$ ) higher compared with women singles (5,03 kg). Male twins are sought after lambing and 4,74 kg also significantly ( $P<0,05$ ) were heavier than female twins (4,05 kg). Overall, singles of lambing had a mass of 5,24 kg and 4,32 kg twins. The difference was statistically significant ( $P<0,05$ ). Final weight (90 days) of male singles amounted to 42,17 kg and 32,75 kg female. On average, singles the three months weighed 36,06 kg and 28,91 kg twins. In both cases the differences were compared values were statistically significant ( $P<0,05$ ). The average weight gain of male singles in the period from 0 to 90 days was 406,24 g and 306,75 g female. The highest increase male singles achieved in the first 15 days (420,67 g), and female, between 0 to 30 days of age (329,57 g). Sex differences in the biggest and average increment between singles were significant ( $P<0,05$ ). Male twins are the largest gain (324,22 g) were between 60 and 90 days and the female (314,02 g) from 30 to 60 days of age. Differences biggest gain of male and female twins, were not statistically significant ( $P>0,05$ ). The average weight gain of male twins at the age of three months was 289,65 g and 250,08 g female. Differences in the average gain of male and female twins were significant ( $P<0,05$ ). The biggest daily gain (354,69 g) singles are achieved from 0 to 30 days of age, the twins from 0 to 90 days (271,39 g). Average daily gain singles during the test period was 344,06 g and 271,39 g twins mentioned.

**Keywords:** sjenicka sheep, birth weight, weight gain, intensive farming

**Introduction**

In the economy of Bosnia and Herzegovina, sheep farming is an important branch of livestock production, and favorable natural conditions make it possible to grow large numbers of sheep (Hodži , 2005). The advantage of the sheep is in the fact that the ruminant and is able certain amount of roughage, different backgrounds and shapes into a high-grade products (Mio et al. 2007). Existing sheep population in Bosnia and Herzegovina in the high percentage of indigenous pramenka makes a large number of strains (Omanovi , 2006). Sjenica improved tuft sheep was named after the village Sjenica the Pešter plateau. Pešter plateau with these sheep, in recent decades, spread to nearly healed western part of Serbia, the northern part of Montenegro, has been found in Bosnia and Herzegovina. Sjenica's sheep is one of the largest domestic sheep with wool C and D varieties (Jovanovi & Savi , 2007). According to some sources this fine assortment of wool sheep have because they participated in its creation Asian sheep with fine wool, which the Turks were reared in the Balkans grew

during his reign. Her breasts are quite deep but narrow chest. Head of sheep is covered with hair which is mostly white with black rings around the eyes, with a black snout lined upper and lower lips and ears with black tips. Of the 100 sheep will be 110-130 lambs. Sjenica's sheep belongs to the group of long-tailed sheep. Late physique a fertility and growth ends at the age of 3-4 years. The sheep are hornless, and the rams have well-developed horns in the form of a spiral (Guti et al. 2006). In our production conditions, of all livestock, sheep farming has been achieved the lowest intensity of development. The reasons for this situation are poor genetic potential, as well as insufficient and unbalanced nutrition some categories of sheep, especially in the winter. In other words, the diet of sheep during this period should be introduced silage or haylage, and a number of other feeds, especially by-products of agriculture and food industry (Handži et al. 1986). The introduction of silage in winter feeding of sheep is one of the most significant changes in order to improve production (Djordjevi & Dini 2003). Antov et al. (2004) suggest that the diet of livestock introduced silage, particularly in sheep that are not milking, breeding offspring in fattening lambs to the higher weight. In order to develop livestock production need to forage on a manifold increase and make cheaper (Fedhofer et al. 1987).

The aim was to establish whether and how the impact of sex and litter size on birth weight and weight gains of lambs Sjenica improved sheep. In terms of the food, sjenicka sheep is destined traditional summer grazing and winter feeding hay with the addition of concentrated feed. Therefore, an additional aim of this study was to recognize the possibility of introduction of corn silage in the diet of sheep during most of the year and determine its production properties.

#### **Materials and methods**

Studies were conducted on a private farm near Visoko on 2011 / 2012th and 2012, / 2013th , during two cycles of lambing ewes. Of the investigation, followed by 35 adult Sjenica improved tuft sheep. Animals were purchased as offspring of repro center Resi i – Rudo. Mothers and, further ancestors examined cattle originating in the Pešter of the most important breeder of the sheep (of the family Bihorac, Milevi , Mujezinovic, Kanjevac, Burovi , etc.). Ewes were first mated with 13-15 months of age. Sheep during the year a minimum of seven months (November-May) fed silage. The rest of the year diet of sheep consisting of meadow hay, with the evening meal of barley or wheat straw. The amount of corn silage during the last two months of pregnancy until just before lambing was about 2 kg / ewe. With silage for the evening meal sheep fed barley or wheat straw. Sheep are for the evening meal until the lambing received by approx. 200 g of concentrate feed (80% wheat bran and 20% grains of barley and oats). Three to four days after lambing, sheep gradually increase the amount of silage to a maximum of 3 kg / ewe per lambing 10-15 days. The evening meal was lambing ewes quality meadow hay at a rate of about 1-1.5 kg and 400 g / sheep concentrate feed (70% wheat bran and 30% barley and oats). Water and mineral supplements in the form of blocks, horses were available ad libitum. Lambs with their mothers continuously resided in the first 15-20 days after lambing. After this period, the lambs were separated from their mothers, with the month of breastfeeding replayed four times a day. At the age of one and a half to two months, until weaning to three months of age, lambs were replayed three times a day to breastfeed. Lambs were separated in special boxes where they were available concentrate feed, water, mineral and vitamin supplements ad libitum. Concentrated feed for the lambs during the first and second months were comprised of: 27.30% ground corn, 32.80% pellets (16% protein), 32.80% bran, 3.80% soybean meal (a by-product in the production of bio-diesel), 3.20% sunflower meal (a by-product in the production of bio-diesel) and 100 g Muvisel's (mineral-vitamin supplement). At a later stage from the second to third month of

concentrated feed for the lambs had the following composition: 50% corn, oats 20%, barley 10%, sunflower meal 10%, wheat 5%, and soybean meal 5%. All components are coarsely ground and mixed. Sheep are a minimum of 24 hours before lambing segregated in special boxes, where they remained together with lambs 7-10 days. Measuring the weight of lambs was performed immediately after lambing, after the sheep lick the lamb. Determining the mass was performed on a digital scale with a precision of 0.00 kg. Measured mass after lambing, with 15, 30, 60 and 90 days of age, when the lambs were rejected by their mothers. During the processing of the results obtained for the purpose of mutual comparison, the lambs were grouped into the following categories: men's singles (MS), single women (ŽS), twin boys (MB), the female twins (ŽB), and total male (ZM) and total female (ZŽ) lambs. During the two-year study measurements were performed on 105 lambs. The average fertility during two pregnancy amounted to 1.5 lamb per ewe. In the first year of monitoring (2011/2012.godina) animals were tested in the first pregnancy, and in the second year, in the second pregnancy. The research results were analyzed using SPSS 16.0. To test the mean values were used Tukey test.

### Results and Discussion

Table 1 shows the weight of lambs shortly after birth until weaning, with three months of age. The results from the above table represent the aggregate average value of two years of research. The highest birth weight (5,61 kg) had a single male lambs. Also, the same category lambs had the greatest final weight (42,17 kg). Significantly lower weight at lambing had a female lambs units (5,03 kg) and total male lambs (5,00 kg). At least they were birth weight in female twins 4,05 kg. From the table it is evident that men's singles achieved a fairly high mass at 90 days, and the average of all male lambs (34,21 kg). Meki et al. (2008) found significantly lower birth weight (3,52 kg) Sjenica's improved sheep. Also, the same authors for the same lambs give a lower weight at the age of 30 days (8,72 kg), 60 days (14,47 kg) and 90 days (20,56 kg). Ramljak et al. (2005) are measuring birth weight lambs Kupres's sheep, found significantly lower values (4,51 kg and 4,36 kg male female). Memiši et al. (2006) found that the birth weight of lambs at bardok amounted to: 3,60 kg single male and 3,30 kg single female. The same authors in their research report that lambs of Bardoka weight at the age of three months amounted to 17,60 kg single male, respectively, 16,80 kg single female. Approximate weight of lambs maternity Tsigai (5,39 kg) was found Antunovi et al. (2012). Meki et al. (2007) point out that the birth weight of lambs in the Svrlijig sheep were 3,82 kg (single) and 2,91 kg (twins).

Table 1. Weight of lambs

Age	Kategorija					
	MS n=12	ŽS n=20	MB n=28	ŽB n=45	ZM n=40	ZŽ n=65
after lambing	5,61±0,38 <sup>a</sup>	5,03±0,16 <sup>b</sup>	4,74±0,25 <sup>c</sup>	4,05±0,14 <sup>d</sup>	5,00±0,24 <sup>b</sup>	4,35±0,16 <sup>cd</sup>
15 days	12,22±0,12 <sup>a</sup>	9,48±0,49 <sup>c</sup>	9,38±0,28 <sup>c</sup>	7,33±0,36 <sup>d</sup>	10,80±0,54 <sup>b</sup>	7,95±0,38 <sup>d</sup>
30 days	17,50±0,28 <sup>a</sup>	14,91±1,64 <sup>b</sup>	11,86±0,68 <sup>d</sup>	10,37±0,30 <sup>e</sup>	13,98±1,02 <sup>c</sup>	11,89±0,77 <sup>d</sup>
60 days	29,86±0,70 <sup>a</sup>	23,43±2,03 <sup>b</sup>	21,08±1,11 <sup>bc</sup>	19,54±1,02 <sup>c</sup>	23,71±1,55 <sup>b</sup>	21,65±1,02 <sup>bc</sup>
90 days	42,17±0,44 <sup>a</sup>	32,75±1,38 <sup>bc</sup>	30,81±1,20 <sup>c</sup>	27,32±1,25 <sup>d</sup>	34,21±1,92 <sup>b</sup>	29,29±1,14 <sup>cd</sup>

MS - single male; ŽS - single female; MB - male twins; ŽB - female twins; ZM - total men; ZŽ - total female

From the table 1 it can be concluded that the sex and number of litters Sjenica improved sheep significantly ( $P < 0,05$ ) influenced the birth and weight of lambs to weaning. Numerous



scientific studies confirm the previous conclusion. Thus, Mio et al. (2003 cit. Mio et al. 2007), Rusty et al. (1995), Jurkovi (2003), Yilmaz et al. (2007) in their research report similar conclusions.

Table 2. The growth of lambs

Days	Category (g)					
	MS n=12	ŽS n=20	MB n=28	ŽB n=45	ZM n=40	ZŽ n=65
0-15	420,67±29,94 <sup>a</sup>	304,83±25,97 <sup>c</sup>	261,67±3,39 <sup>d</sup>	220,13±19,07 <sup>e</sup>	341,17±30,58 <sup>b</sup>	244,33±17,62 <sup>de</sup>
0-30	396,56±4,31 <sup>a</sup>	329,57±46,08 <sup>b</sup>	237,77±16,41 <sup>d</sup>	209,00±9,19 <sup>e</sup>	297,31±28,01 <sup>c</sup>	232,46±15,86 <sup>d</sup>
15-30	369,00±18,78 <sup>a</sup>	277,42±46,03 <sup>b</sup>	218,83±43,03 <sup>c</sup>	183,96±11,75 <sup>e</sup>	293,92±34,91 <sup>b</sup>	219,22±19,05 <sup>c</sup>
0-60	404,25±16,76 <sup>a</sup>	310,56±29,40 <sup>b</sup>	272,37±15,77 <sup>de</sup>	256,23±16,04 <sup>e</sup>	311,93±23,25 <sup>b</sup>	295,28±13,65 <sup>c</sup>
30-60	411,94±29,21 <sup>a</sup>	328,40±14,99 <sup>bc</sup>	314,87±22,52 <sup>c</sup>	314,02±30,87 <sup>c</sup>	351,27±22,99 <sup>b</sup>	319,55±21,62 <sup>d</sup>
0-90	406,24±5,83 <sup>a</sup>	306,75±10,78 <sup>c</sup>	289,65±11,68 <sup>d</sup>	250,08±13,75 <sup>e</sup>	324,63±19,57 <sup>b</sup>	275,84±11,36 <sup>d</sup>
60-90	410,22±19,17 <sup>a</sup>	245,53±9,01 <sup>d</sup>	324,22±8,84 <sup>c</sup>	200,11±18,12 <sup>e</sup>	350,02±15,28 <sup>b</sup>	220,75±12,28 <sup>e</sup>
30-90	411,08±6,90 <sup>a</sup>	286,97±9,55 <sup>d</sup>	317,72±15,24 <sup>c</sup>	273,46±21,17 <sup>d</sup>	352,73±18,18 <sup>b</sup>	279,60±12,68 <sup>d</sup>

MS - single male; ZS - single female; MB - male twins; ŽB - female twins; ZM - total men; ZŽ - total female

Average daily gain of lambs Sjenica improved sheep from 15 days of age until weaning (90 days) is shown in table 2. It is evident that the male lambs had higher ( $P < 0,05$ ) daily gains compared with female. Also, differences were present ( $P < 0,05$ ) and within the same sex and of different types (single or twin). It is important to note the high daily gains in male single who were highest in the first 15 days (420,67 g/day). Female lambs (single) the highest daily gains achieved in the first month of age (329,57 g/day). On the other hand, at least daily gains of lambs were between the fifteenth and thirtieth day (218,83 g/day male twins and female twins 183,96 g/day). It is evident that on the average daily gain sex and type of lambs had statistical significance ( $P < 0,05$ ).

Table 3. Comparative review of maternity and weight gain single and twins

Age	mass at lambing		daily gains	
	Singles n=32	Twins n=73	Singles n=32	Twins n=73
after lambing	5,24±0,19 <sup>a</sup>	4,32±0,15 <sup>b</sup>	-	-
15 days	10,39±0,67 <sup>a</sup>	7,67±0,39 <sup>b</sup>	343,44±33,02 <sup>a</sup>	227,05±17,18 <sup>b</sup>
30 days	15,89±1,09 <sup>a</sup>	10,87±0,37 <sup>b</sup>	354,69±32,59 <sup>a</sup>	218,59±9,43 <sup>b</sup>
60 days	25,57±1,62 <sup>a</sup>	20,26±0,82 <sup>b</sup>	341,79±25,05 <sup>a</sup>	263,76±12,19 <sup>b</sup>
90 days	36,06±1,94 <sup>a</sup>	28,91±1,14 <sup>b</sup>	344,06±19,62 <sup>a</sup>	271,39±11,65 <sup>b</sup>

In the table 3 presented comparative values maternity weight and daily gain singles and twins. It is evident that singles of the same age had a higher weight ( $P < 0,05$ ) and had significantly ( $P < 0,05$ ) greater daily gains than twins. The conclusion of the preceding sentence is consistent with research Notter et al. (1991), Greef et al. (1992), Nawaz and Meyer (1992), Owen (1996).

### Conclusion

Exploring the effects of sex and litter size at birth and weight of lambs at the age of 15, 30, 60 and 90 days, it can be concluded that:

1. The average weight of male lambs at lambing was 5,00 kg and females 4,35 kg. The differences found in the effects of sex and type of litter size were statistically significant ( $P < 0,05$ ).
2. The final average weight of male lambs at weaning (90 days old) has been 34,21 kg and 29,29 kg female. The differences are also due to the impact of these factors were statistically significant ( $P < 0,05$ ).
3. The highest average daily gain (420,67 g/day) were determined in male singles in the first 15 days. On the other hand, the lowest average daily gains were established in female twins between 15 and 30 days of age (183,96 g/day). The impact of sex and type of litter had statistical significance ( $P < 0,05$ ).
4. During the two-year study using corn silage in the diet of Sjenica improved sheep showed quite reasonable. Neither sheep during feeding cycle is not had any health problems In addition, during the use of silage in sheep suckling period did not significantly lost their shape, which certainly contributed to the relatively high fertility in cattle (150%). In economic terms silage also confirmed all its advantages compared to other forages.

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## IMPACT OF HONEY BEES ON POLLINATION OF SOME PLUM VARIETIES

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### Abstract

Under the project No.31063, which was funded by the Ministry of Education, Science and Technology Development of the Republic of Serbia, a study was conducted on the Experimental Field *Radmilovac* of the Faculty of Agriculture, University of Belgrade. Pollination of different plum varieties was observed, including: *Toper*, *Lorida*, *Avalon* and *a anska rodna*. The aim of this study was to determine the impact of honey bees on the rate of pollination, fruit setting and yield of the known and new plum varieties.

Three plum trees of each variety were selected, and three budding branches on each tree were isolated. Three types of pollination were planned and executed: by wind - anemophily, where branches were isolated by tulle bags; self-pollination, where branches were isolated by pergament paper; and entomophily by honeybees, where the branches with buds and flowers were naturally pollinated. During the spring, there was a continual counting of flowers, set fruit, fruits remaining after drop and at the time of picking (when ripe). The recorded data were then statistically analyzed.

For *a anska rodna*, it was found that in case of self-pollination, the number of fruit on trees just before the June fruit drop was 20% and 10% at the time of picking; when anemophily was applied, 49% remained before the fruit drop and 15% at time of picking; in case of entomophily, the rate of remaining fruit before drop was 44% and 28% were finally picked. For *Toper* variety the results were the following: self-pollination – before fruit drop 20% remained, and 3% were picked; anemophily - 14% before the drop and 8% were picked; entomophily by honeybees - 33% before the drop and 26% at the time of picking. The picking rate for *Avalon* was: 1.2% in case of self-pollination, 7% where anemophily was used, and 28% in case of entomophily. *Lorida* variety showed the following picking rates: self-pollination - 13%, anemophily - 53%, and pollination by bees - 25%.

**Key words:** plum, pollination, *a anska rodna*, new varieties

### Introduction

Phylogenetically speaking, a plum belongs to the same genus (*Prunus*) as almond, peach, apricot, sour cherry, cherry, bird cherry and *džanarika*, which all have a hard stone pit. What makes this subgenus *Prunus* different from other subgenera (Mladenovi et al., 1996) are solitary terminal and lateral buds as well as smooth pit (Bulatovi , 1991). Stone fruits are in the group most commercialized continental fruits, as they account for 65% of total fruit production (Šoški , 1991). Plum still holds the title of a „queen“ in Serbian fruit growing. The dominant variety used to be *Požega a*, but in the last few decades more and more new varieties have been introduced, *Stenli* and new varieties from a ak being the commonest (Gvozdenovi et al., 1997).

Due to early, fast and explosive blooming, almost all plum varieties need honeybees as pollinators, regardless of the fact that some varieties indicate certain level of self-pollination capacity (Miši , 1996; Stankovi et al., 1990). The main problem represent the different

maturing time of male and female reproductive organs in the same flower and physiological incompatibility between pollen and stigma. The imperative in cross-pollination is not only the transfer of pollen from a different plant, but also pollen from other varieties of the same species. During the evolution process, honeybee developed the highest capacity for transfer of pollen from different sources as on its body hair several million of pollen grains can be found.

In fruit growing today, the participation of honeybee in pollination is steadily increasing, because there is no fertilization without transfer of pollen grain to stigma (Miši ,1996). The honeybee is the most important of all natural pollinators (solitary bees, wasps, bumblebees, some species of ants, etc.), as it accounts for pollination of 80% of fruits, and even 95% in intensive fruit growing ( erimagi , 1985). Such a high share of honeybee participation in pollination results from the fact that, due to the use of pesticides in orchards, almost all spontaneous pollinators have been eradicated, while the use of modern agricultural machinery, together with deep winter ploughing and frequent spraying destroys cocoons of other insects and pollinators. The advantage of honeybee over other pollinators lies in multiple visits to the same flower (as long as it has nectar, pollen and etheric oils), that is, until the fertilization becomes certain, after which the flower ceases to be attractive and interesting to a bee. It can be concluded that honeybee is an insect which created the highest level of symbiosis with plants. It is a mutually beneficial state, both for a plant and a honeybee, as they cannot sustain without each other as well as the present agriculture (Mladenovi ,1987).

Honeybee, as a polytropic insect, exerts this extremely important role, among other things, thanks to one of its characteristics – it does not mix pollen of various fruit species, but only of varieties (Stankovi & Jovanovi , 1990).

Honeybee instinctively collects nectar only from one species of fruit and plants (Simi et al., 1995). Different plant species show fluctuations in quantity of nectar secretion during a day, even an hour. When one species stops nectar production, honeybee moves to another one which offers nectar at that moment and with higher content of sugar. Therefore, during a season the honeybee visits several different plant species, which makes it the best and most reliable pollinator.

With some minor number of exceptions, entomophily is most common way of pollination of fruit cultivars. It is absolutely essential for optimum yield of self-sterile species and varieties of fruit, such as is sour cherry (Mladenovi & Peši , 1996), but it also contributes to high and certain yield of self-fertile species and varieties. Presence of honeybee colonies in pollination of cultivars has positive effects on explosive flowering, especially in case of stone fruits such as are the studied varieties. Pollen transfer activity of honeybees is affected mostly by meteorological conditions. During fruit tree flowering, even a day or two without rain are enough for good effects and when the temperature in shade is above 12°C (Mladenovi , 2011).

When using honeybees as pollinators in intensive fruit growing, the problem is how to protect them from toxic chemicals which are used for protection of fruit trees from diseases and pests (Mladenovi et al., 2013). A special care should be taken not to do spraying during flowering, or not to allow bees to leave the beehive if the spraying is necessary ( erimagi ,1985).

Depending on the hereditary factors and meteorological conditions, flowering of all varieties of plum takes place in March and April. According to the start period and sequence of flowering, all plum varieties can be divided into: early-blooming, early-mid blooming, mid-late blooming, and late-blooming. The process of flowering of stone fruits is much faster and stronger than in pome fruits, and depending on the variety and weather conditions the flowering lasts between 6 and 12 days. The plum flower is hermaphrodite and complete. It

consists of 5 sepals, 5=7 petals, 16=19 stamens and 1 pistil. Flowers are single or form clusters (Šoški , 1991).

The objective of our experiment was to compare the impact of honeybee as a pollinator in comparison to anemophily and self-pollination. This was done by counting and recording the number of fruits 60, 80 and 100 days after fertilization and at picking stage (ripe).

### **Materials and method**

The study was conducted at the experimental field Radmilovac of the Faculty of Agriculture, University of Belgrade. The plum orchard included mix of different domestic varieties and newly introduced ones which are actually subject to study presently. The plantation of plums is at the height of 135 m a.s.l. and the basic variety is *džanarika* (*Prunus cerasifera*). The cultivars' shape is pyramidal.

The soil type was gajnjacas. During the experiment, it was in the state of idle land, with regular application of mineral fertilizers. The soil was treated with agrotechnical measures every year and the protection from diseases and pests was also regular.

The varieties used in the experiment were: *a anska rodna*, *Toper*, *Lorida* and *Avalon*, and they were monitored from the flowering phenophase to ripening phenophase and fruit picking. For pollination of the selected varieties 50 honeybee colonies located at 300 m distance were used. The beehives were of a modern LR type, the ownership of the Faculty of Agriculture.

Variety *a anska rodna* has been exploited in our country since 1965, when it was created as a hybrid resulting from interbreeding between *Stanley* and *Požega a*. It gives high yield and it is suitable for drying, processing and consumption in a raw state. The flesh part varies from 26 to 30 g. Its skin is of blue colour and has ample epicuticular wax coating ("wax gloom"). Its flesh is juicy, yellowish, of superb nutritional quality. The size of its stone is medium to large and it is compact. It can be easily separated from flesh part. It requires regular and severe pruning in order to have quality fruits and avoid alternate bearing. The plum tree is not so dense, which makes it suitable for dense planting. Plum trees are adaptable to a wide range of climates. Picking season is late August. It is susceptible to powdery mildew and plum rust, as well as sharka (plum pox virus).

The other varieties - *Toper*, *Lorida* and *Avalon* are still in the phase of studying, so more information about the results will be available in the future.

A study was conducted in order to determine the impact of honeybee as a pollinator of the plum varieties *a anska rodna*, *Toper*, *Lorida* and *Avalon*, through comparison with anemophily and self-pollination. Three fruit trees of each variety were selected, with budding branches of various types and at different locations in the crown. The number of buds was established by counting, then number of pollinated flowers, number of set fruit, and the fruit drop was recorded 60, 80 and 100 days after fertilization, and finally the number of picked fruits was counted.

### **Results and discussion**

The results are based on a two-year study of the impact of honeybee on transfer of pollen in plum varieties *a anska rodna*, *Toper*, *Lorida* and *Avalon*. They indicate that the number of set fruit until picking season varies depending on the type of pollination.

Table 1. Rate of set and picked fruits in variety *a anska rodna*

Pollination type	Set fruits	Fruit drop			Ripe	Percentage
		After 60	After 80	After 100		
Entomophily	148	68	54	44	41	from 44%
	83	32	21	21	21	up to 28%
	92	42	31	28	28	
Anemophily	77	11	8	5	5	from 49%
	45	45	16	13	13	up to 15%
	52	30	9	9	8	
Self-pollination	28,3	3	2	2	2	from 20%
	23	1	1	1	1	up to 10%
	42	15	7	7	6	

The table 1. shows that the number of set fruits until June fruit drop was the largest in case of entomophily by honeybees (44%) and anemophilic pollination (49%). Number of ripe and picked fruits was the biggest in case of entomophily - 28%, where the anemophilic pollination resulted in only 15% and self-pollination in 10%. All three pollination types resulted in the rate of picked fruits over 8%, which is desirable for plantations, but it is evident that entomophily is the most reliable type of pollination for the variety *a anska rodna*, and that is two times more in comparison to self-pollination and almost 100% in relation to wind pollination. The obtained data are in line with those found in literature.

Table 2. Rate of set and picked fruits in variety *Toper*

Pollination type	Set fruits	Fruit drop			Ripe	Percentage
		After 60	After 80	After 100		
Entomophily	96	10	10	10	10	from 33%
	86	58	58	58	56	to 26%
	129	34	16	16	16	
Anemophily	88	7	0	0	0	from 14%
	47	10	7	7	7	to 8%
	50	9	9	8	8	
Self-pollination	25	0	0	0	0	from 20%
	51	19	4	4	4	to 3%
	40	4	0	0	0	

Table 2. shows the dynamics of fruit drop until ripening. Entomophilic pollination resulted in the largest number of fruits until fruit drop (26%). Also, the rate of picked fruits was highest in case of pollination by honeybees (26%); for wind pollination that rate was 8% and for self-pollination 3%. It can be concluded that the honeybees are necessary for pollination of the variety *Toper* in order to achieve profitable production.

Table 3. Rate of set and picked fruits in the variety *Lorida*

Pollination type	Set fruits	After 60	After 80	After 100	Ripe	Percentage
Entomophily	31	31	31	27	27	Od 63%
	99	45	26	13	12	Do 25%
	84	58	44	18	15	
Anemophily	32	32	32	30	30	Od 72%
	24	10	5	3	3	Do 53%
	40	27	21	18	18	
Self-pollination	17	0	0	0	0	Od 35
	33	21	8	6	6	Do 13%
	18	3	3	3	3	

Table 3. shows that the rate of survival of set fruits until the June fruit drop depends on type of pollination. In case of anemophilic pollination, this rate was the best (72%), as well as the rate of picked fruits. It could be concluded that the variety *Lorida* is partly self-fertilizing and can be grown with success without involvement of reliable pollinators.

Table 4 – Rate of set and picked fruits in the variety *Avalon*

Pollination type	Set fruits	After 60	After 80	After 100	Ripe	Percentage
Entomophily	109	65	35	31	31	Od 66%
	112	96	42	37	37	Do 28%
	98	47	20	19	19	
Anemophily	79	49	4	3	3	Od 52%
	93	49	16	15	14	Do 7%
	57	31	0	0	0	
Self-pollination	25	11	1	1	1	from 28%
	30	17	0	0	0	to 1.2%
	27	10	0	0	0	

However, Table 4 indicates that for variety *Avalon* the presence of honeybees is necessary, because not only that the number of remaining fruits before drop was the highest (66%), but also the number of picked fruits (28%), as for entomophilic pollination it was 7% and for self-pollination 1.2%.

### Conclusion

The results of the study on impact of honeybees on pollination of plum varieties *per*, *Lorida*, *Avalon* and *a anska rodna* from the aspects of fruit setting, fruit drop and number of picked fruit, the following can be concluded:

1. Honeybee (*Apis mellifera* L.) is the most numerous, most reliable and most complete pollinator of stone fruits.



2. The studied varieties *Toper*, *Lorida*, *Avalon* and *a anska rodna* have the highest rate of fertilized and picked fruits when entomophilic pollination is present (28%) as shown in comparison to other types of pollination, except for variety *Lorida* (53%).
3. The highest intensity of fruit drop occurs in the first period after flower falling off.
4. Involvement of honeybees in modern fruit growing is the cheapest pomotechnical measure, if compared to other pollinators and types of pollination.

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## SPRING DEVELOPMENT OF BEE COLONIES IN THE AGRO-ECOLOGICAL CONDITIONS OF THE WESTERN BOSNIA AND HERZEGOVINA

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### Abstract

The aim of this study was to investigate the effect of fruit flow on commercial beekeeping using several pastures in western Bosnia and Herzegovina (BH) moving bees to different altitudes and different honey plants.

The experiment was carried out on the half-stationary apiary of Rade Bauk near by Drvar on the 460 m of height above sea level. The aim of the paper was to examine influence of the fruit pasture on commercial beekeeping using a few pastures in west BH by moving bees to different heights above sea level and to different melliferous plants. Bees spend winter in Drvar where the apiary is located in winter where they use fruit-developing pasture, and in Rore at 950 m above sea level bees use meadow and heather pasture, and in autumn bees are moved again to Drvar in the winter station. All colonies were carefully examined, prepared for wintering, and meteorological parameters are carefully analyzed including critical points for bees.

Bee colonies, which were prepared for winter with plenty of pollen were stable wintered and developed in the spring faster, regardless of the input of pollen from early blossoming plant species. Fruit pasture is irreplaceable developing pasture with a big influence on development of brood nest as well as quantity of honey in the next bee pasture. Bee colonies can take advantage of meadow and heather pasture if we continue stimulated feeding and introduction of two-queen way of beekeeping.

**Key words:** bee colonies, spring development, brood

### Introduction

Beekeeping is a specific agricultural branch in Bosnia and Herzegovina where Domestic Carnica is bred (*Apis mellifera carnica* Poll.).

In order to exploit bee colonies in the best possible way in production of direct products (honey, royal jelly, pollen, wax, propolis, bee venom), also for pollination purposes, it is necessary to develop them by adequate apitechnic measures to their maximum and also make the best possible use of them. Apart from the timely preparation of bees for wintering, it is necessary to provide optimal spring development by different apitechnic measures (Bel i et al., 1985). Early spring development is of the crucial importance especially for exploitation a few main bee pastures which are recently more demanding for western parts of Bosnia and Herzegovina.

Spring development is a specific period for both honey bees, and also for each beekeeper because at that time the most important stimulative measures are usually done for strengthening bee colonies. Most attention is paid on bee queens stimulation in order to lay more eggs because from that brood bees, who take part in the exploitation of almost all main pastures in western Bosnia and Herzegovina, are developed. For that purpose it is necessary to have considerable amount of honey, and intake of fresh nectar and pollen as well (Sahinler, 2003). In the beginning of spring development it is usual that the surface of honey

and pollen on combs is reduced because of the stronger nutrition of bees and brood, but with inflow of the fresh nectar and pollen, the balance is made between brood and food reserves (Kulin evi , 2009; Mladenovi et al., 1999). Because of that it is very important to define critical points for stimulation of bee colonies and right intervention with sugar-honey cakes, sugar syrup or crystal sugar. Supplementary feeding with honey is avoided, not only because of the economic reasons, but also because of the danger to spread some bee diseases (Todorovi et al., 1983; Tosi et al., 2012).

At big apiaries a number of apitechnic measures are simplified and they are done serially, not to go into details for each bee colony (Crailsheim, 2010; Dietz et al., 1975; Kaftanoglu, 2007). Because of that it is important to make decision and expand brood chamber, or honey chamber if necessary, or stimulate a bee queen and bees to be more active, prevent a brood cold, do water supply, etc by visual symptoms of bees at the entrance and quick examination of brood chambers (Mladenovi et al., 1996, 1998, 2002). With LR bee hives it is usual to work with chambers as a working unit and all expanding or contracting are done by adding or taking away chambers.

Specific conditions of keeping bees in Bosnia and Herzegovina with specific melliferous plants from blossoming of *hazel*, *cornel*, *stone-fruit* and *apple fruit*, *broom*, *sage*, *acacia*, different *meadow melliferous* plants till heath, give possibilities for good spring development of colonies but also extracting of honey surplus and other bee products (Bertazzini et al., 2010). Because of that spring development is a big challenge for producers, as well as for scientists in order to define the best apitechnic measures regarding biological development of colonies.

### Materials and methods

Experiment was carried out in the apiary with 350 bee hives belonging to a beekeeper Rade Bauk in Drvar at 460 m above the sea level. In springs 2010 and 2011 apiary used *fruit pasture*, and in the first half of May it was moved to the village of Pope which is situated 30 km far from Drvar, towards Glamoc, at 950 m above the sea level. At this location bees used *meadow* and *heather pasture*.

#### *Picture 1. Winter apiary in Drvar*

Bee colonies are kept in LR – Langstrot-Rut ten-frame bee hives. This hive is most-widely used in the world, and the biggest advantage of this hive is that brood nest and honey chambers are easily broaden or narrowed by adding or taking away chambers. Carniola bees are used in bee hives at this apiary (*Apis mellifera carnica* Poll.). Main characteristics are grey rings covered with whitish hairs. In spring it develops fast till its strength, and because of that it gets the instinct to swarm naturally. On the combs it is tranquil, mild temperament, it also fights for its brood well, it is not disposed to robbery, it produces small quantity of propolis. It also winters well even in bad weather conditions. It has the biggest capacity of the honey sac (0.02 to 0.04 mm<sup>3</sup>) and the longest tongue (6.5 to 8.2 mm, *in vivo*).

Bee colonies were examined in February 2010/2011, when we found out the number bee ways covered by bees, food quantity they have and we started stimulation by adding bee cakes (1kg) with nozucid.

The second examination was done at the end of March 2010/11. when we found out, apart from the previous information, brood surface and quantity of pollen in bee colonies.

The third examination was done at the end of April 2010/2011. when the surfaces of brood, honey and pollen were identified. During this examination at well-developed colonies

we rotated brood chamber for 180°, changed position of brood chambers and chambers below brood chambers, one more bee cake (1 kg) `stimular` was added to each colony.

The fourth examination was done on the 10<sup>th</sup> May 2010/2011. when the surfaces of brood, honey and pollen were defined. One chamber was added to all colonies that fulfilled the space inside bee hives.

All collected data was recorded, statistically processed and later discussed.

## Results and discussion

### Analysis of brood surface

Table 1. Analysis of the brood surface shown in dm<sup>2</sup>

Date	H I V E S										Middle value
	16	73	42	117	28	55	92	84	107	191	
<b>03.2010/11</b>	55	57	66	32	30	67	24	31	59	41	<b>46,2</b>
<b>04.2010/11</b>	92	108	106	116	98	103	72	74	83	99	<b>95,1</b>
<b>05.2010/11</b>	110	112	118	134	140	124	88	102	117	132	<b>117,7</b>
<b>Difference</b>	55	55	52	102	110	57	64	71	58	91	<b>71,5</b>

Based on the data from the Table 1. it is seen that the brood surface from the examination carried out in March 2010/11. until the examination carried out in May 2010/11 was constantly increasing. Brood surface in 45 days increased for 71.5 dm<sup>2</sup>, what showed that colonies that were well-prepared for wintering with adequate food storage to faster development in spring.

For most observed colonies more quantities of honey and pollen were left in autumn, and frames with honey were added to colonies that showed the lack of food during the first spring examination and stimulation with sugar-honey cakes started.

Good meteorological conditions influence fast spring development apart from other factors. In both examined years there were no critical temperatures which would prevent bees to keep optimal temperature of brood, so there was no discontinuation in development. Favourable temperatures were also present during blossoming of cornel. There was a plenty of cornel in the area of Drvar. It usually blossoms around 20th March, which classifies it in the main stimulative early nectar pasture. A special stimulation of the bee brood happens in the period of blossoming of stone-fruit as well as apple fruits.

In 30 days of spring development in the period from the end of March till the end of April brood surface doubled (from 46,2 to 95,1 dm<sup>2</sup>, and for the next 15 days brood development also increased for 22 dm<sup>2</sup>. In some colonies (beehive 28) that development increased for 30 dm<sup>2</sup> in March, to 98 dm<sup>2</sup> in April, and to 140 dm<sup>2</sup> of brood in May.

### Analysis of honey surface

Table 2. Analysis of honey surface

Date	H I V E S										Middle value
	16	73	42	117	28	55	92	84	107	191	
<b>03.2010/11</b>	24	98	104	32	112	96	56	80	24	96	<b>72,2</b>
<b>04.2010/11</b>	80	80	96	72	82	64	64	88	64	80	<b>77,0</b>
<b>05.2010/11</b>	88	88	120	104	120	98	80	88	72	92	<b>95,0</b>
<b>Difference</b>	64	-10	16	72	8	2	24	8	48	-4	<b>22,8</b>

Based on the data from the Table 2. it is obvious that surface of honey between first two examinations there was an insignificant increase ( 4,8 dm<sup>2</sup>), it means that nectar intake

was bigger than honey consumption for the brood nutrition in that period. *Cornel* and *dzanarika*, a kind of a stone-fruit are most responsible for this increase. Honey surface between the second and the third examination increased for 18 dm<sup>2</sup>. The total growth of honey surface between the first and last examination was 22,8 dm<sup>2</sup>.

It is usual for this area that the honey surface was reduced until the blossoming of fruit, and during fruit pasture it had an insignificant increase or stayed at the same level. Apart from the increase of the honey consumption for the brood nutrition, this happens also because of the changeable meteorological conditions and insufficient exploitation of nectar and pollen pasture. Because of that two enriched bee cakes with vitamins and growth gormons were added.

### Analysis of pollen surface

Table 3. Analysis of pollen surface

Date	H I V E S										Middle Value
	16	73	42	117	28	55	92	84	107	191	
<b>03.2010/11</b>	18	27	26	25	27	25	18	14	18	24	<b>22,2</b>
<b>04.2010/11</b>	24	36	30	27	18	12	8	9	11	16	<b>19,1</b>
<b>05.2010/11</b>	32	41	33	27	41	31	22	24	21	31	<b>30,3</b>
<b>Difference</b>	<i>14</i>	<i>14</i>	<i>7</i>	<i>2</i>	<i>14</i>	<i>6</i>	<i>4</i>	<i>10</i>	<i>3</i>	<i>7</i>	<b>8,1</b>

Taking into consideration the data from the Table 3. it is obvious that between first two examinations surface of pollen was reduced for 3.1 dm<sup>2</sup>. This reduction of the pollen surface is the result of the intensive growth of the brood for which nutrition it is necessary to use large amounts of pollen. Between the second and the third examination there was a significant growth of the pollen surface which was 11.2 dm<sup>2</sup> although in that period there was an intensive growth of the brood surface. Fruit pasture had the biggest impact on this growth which gave, apart from nectar, a lot of the pollen powder.

Comparing data from the first and the third examination, it is seen that the surface of brood was increased from 8.1 dm<sup>2</sup>.

Based on the data from the previous tables it can be concluded that bee colonies which had bigger reserves of pollen in March, with the condition that they also had enough honey, had more intensive spring development than the bee colonies that had less pollen and honey.

### Conclusion

Based on the experimental work on spring development of bee colonies in the agroecological conditions of west Bosnia and Herzegovina, it can be concluded:

- Bee colonies which were wintered in autumn with more bee ways, bigger quantities of honey and pollen, had more intensive spring development than the bee colonies which were weaker.
- Brood development for 40 days of spring development ranged from 30 dm<sup>2</sup> to 140 dm<sup>2</sup> what was of crucial importance for exploiting of meadow and heather pasture.
- Surface of honey in the examination period increased in average for 22.8 dm<sup>2</sup>, which had a stimulating effect on preparation of colonies for the next meadow pasture.
- Surface of pollen was reduced in the early spring, regardless the increased intake, from 22.2 dm<sup>2</sup> to 19.1 dm<sup>2</sup>, but blocking the brood it was increased to 30.3 dm<sup>2</sup> in the second phase of development.
- In this region meadow pasture is the first main pasture from which honey harvest is expected, so beekeepers should feed bees stimulating them and to perform double-queen beekeeping, preparing colonies for this pasture.

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## **SAFETY OF BEESWAX PRODUCTIONS**

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### **Abstract**

Beeswax is the second most important product in beekeeping industry. It secreted from four pairs of wax glands on the ventral (lower) side of the abdomen (belly) of the workers. The secretion occurs in bees that are about two week old and resulting reduction of sugar synthesis of digestive origin. The aim of this paper was to determine the chemical, physical, and organoleptic properties of beeswax, which could affect on its safety. Beeswax is a very stable substance under normal conditions and retains its characteristics, but under certain circumstances, it may still have some modifications and defects may occur. Due to high demand and low production in some countries, there is the rising need for import and counterfeiting of beeswax. Increased production of beeswax becomes important, as well as the production of honey and have implemented modern technology for wax. Technology of wax includes annual replacement of old honey combs, carefully collecting all laterals, construction and building material amputation, carefully collecting scrap of wax from the hive floor coverings, regeneration frames. In terms of safety beeswax explored the factors that influence on the quality and quantity of beeswax, the way of purification combs, beet, industrial processing of wax. Safety of beeswax often threatens wax pests.

**Key words:** beeswax, wax glands, technology acquisition

### **Introduction**

Researches (Whitcomb, 1946) have shown that the amount of sugar consumed by the bees to produce 500gr wax varies in some bee colonies from 3.0 kg to 4.0 kg with an average of 3.8 kg. Pure beeswax as it is flaked after being excreted by bees is obligatory white regardless of whether the bees are fed with dark honey or sugar syrup. Yellow tint in the wax comb, resulting from carotene pigments derived from pollen (Vansell and Bisson, 1935,; Tisher, 1940). Combs that play the litter darken after prolonged use due to the accumulation of parts jacket bee pupae that remain after performing in cells. The bright color of wax is more appreciated than dark-colored wax (Bogdanov, 2004). The composition of beeswax depends on the content of paraffin hydrocarbons, free fatty acids, esters of fatty acids and fatty alcohols (Tulloch, 1980). The mass used gas chromatography detector, for detecting falsification wax (Apimondia, 2009). Archeological discoveries in a cave in Slovenia show that the beeswax used in dentistry for more than 6,500 years, found the bone of the human jaw with a tooth whose cavity is filled with beeswax ([www. huffingtonpost.com](http://www.huffingtonpost.com), 2012).

### **Literature review**

Chemical composition of pure beeswax thoroughly investigated one Australian study group (Downing et al, 1961). It was found that beeswax consists of 16 % hydrocarbons (C21 to C33 odd numbers), 31% straight-line monohydrate alcohols (C24 to C36 even numbers), 3 % linear monohydrate alcohol (C24 to C32), 31% acid (C12 to C34, mainly C16), 13 % hydroxyl acid (C12 to C32 , mainly C16), and 6% of other substances. Further analysis of the



hydrocarbon fraction of beeswax by gas chromatography (Streibl et al, 1966) indicated the presence of two homologous series of paraffin's and olefins 6 homologous series. For further interest in the study, should be of excellent study done by the Callow (1963), where the corrected certain inconsistencies and errors in interpreting the results of earlier research. Physical properties of pure beeswax that has a specific gravity of 0.95 and an aroma that is similar to honey and weakly expressed distinctive taste. It melts at 64, 4°C, and hardens to 63, 4°C and a density of 20°C (Vansell and Bisson, 1940). The scope of these three values was 62-65°C, 60, 6 to 63, 4°C and from 0,939 to 0,987. Beeswax does not dissolve in water, very slightly soluble in cold alcohol and completely dissolved in volatile oils, chloroform, ether, benzene at 30°C, and carbon disulfide at 30°C. During storage at low temperatures beeswax often develop a powdered substance on its surface, which is called the flour, it is not musty, as some beekeepers believe. It is not clear what is causing this, but it can be seen under a microscope, the characteristic crystal structure. Melting point of the powder surface wax is 39°C, which is far from those of wax (Vansell and Bisson, 1940). Organoleptic qualities that wax usually meet in the form of molded blocks that need to have a homogeneous structure, free of impurities inside or without porous mass at the bottom of the block and without emulsion zone or remains saponification. The fracture has the appearance of crystals. The shape and structure depends on the quality of the starting material and the cooling time and conditioning. White wax, extra class that is obtained by melting wax caps from constructional or laterals has a homogenous structure with a fine grit. Wax, which has obtained from old, combs a crystal structure with a granular medium. Plasticity beeswax is quite specific. Pressure between your fingers to soften, easily modeled and becomes sticky. When pressed into a thin film, becomes homogeneous and transparent. Color depends on the quality of the starting material, the technology of extraction and purification. Color is a characteristic feature for determining quality. White color is valid for top quality, yellow to black for the first of thirds. Smell the wax is specific, similar to the smell of warm honey, but it depends on the extraction process. Smell the fat shows the presence of seoul or sterin, the smell of kerosene indicates the presence kerosene etc. Organoleptic characteristics are generally sufficient to assess the quality and origin of wax, and if this is not possible because of the physic- chemical analysis.

#### Technology of production of beeswax:

By finding the hive with movable comb, beekeepers were forced to come to the breaking up of the majority of honeycomb, so all that wax melts, and received substantial amounts of pure wax. After that, using modern hives and beekeepers centrifugation frames back most of the honeycomb in the hive so that the only part of mostly old frames melts and gets a small amount of wax. Since it is a small part of beekeepers producing wax is estimated that each hive produces 200-300g of wax, which is well below the need for beekeeping, let alone for other purposes. Due to the large deficit raises the urgent question of purity and quality of the wax in the form of honeycomb to the health of bees and the impact on quality of bee products. The fact that the uncontrolled quality of input materials in hourly basis, and the quality of honeycomb, showing how much danger beekeeping. It is necessary to break the conventional opinion that the production of wax reduces the production of honey in the hive. Beekeepers stimulate natural instincts of bees that disgusted litter and collect food reserves and neglected instinct to build honeycombs. Experience shows that there is a strong correlation between the intensity of nectar intake (pasture) and production of wax. A bee can produce 500g of wax and raise 26,000 larvae (V.Taranov) so that it takes on a real production capacity of wax. Production of wax, especially dependents on the amount of pollen in the hive that helps wax gland activity. Instinct construction is particularly pronounced when it is parallel with the presence of good pasture with raising a large brood, we have enough heat in

the hive, and enough space for the construction (frames with hourly basis). Knowing the factors that affect the production of wax in the hive beekeeper can increase production through the following measures: Annual replacement of old frames (min1/3), carefully collect all the laterals, construction, and amputation building material (biological fight against varroa), carefully collecting debris from the hive floor coverings, regeneration frames (deeper tilting honeycomb).

Cleanses wax:

Cleaning operation or refining wax has done by cleaning wax from foreign bodies. The method consists of repeated melting wax in soft water and cooling by precipitation with cleaning the bottom of a block of wax. The melting temperature should not exceed 90°C. In this it should be noted that many waste in wax having a specific gravity close wax and precipitated after 48-72 hours, and sometimes more. For this reason it is necessary to ensure slow cooling wax in a warm room with a well- insulated court in which the wax cools. The wax should melt in enameled or aluminum. The Court should be clean and at the bottom there is 2-3cm soft water or rainwater. When the wax is melted off the court with the heat and well drowns and leave to cool to room temperature. After this cooling wax in the form of a block can be easily removed from the mold and clean the various supplements on the underside of the block. This procedure wax becomes brighter colors and with fewer additives. When melting honeycomb extra class (even eating my wax and virginal honeycomb). The purification process is not necessary, if properly carried out extraction. Remnants of wax are the rest of the first melting honeycomb, in the extraction process, which still contains a certain amount of wax. It is assumed that in our remnants of wax contains 40% wax. With the use of powerful industrial presses pressed to install processing solvents can wax content reduced to only 3-4% that cannot get away, the rest of the otherwise good fertilizer. In order to obtain a good quality wax remnants of wax, it must be well dried in pieces that are not too large and placed in bags that they would not be attacked by moths or mildew. Should be free of other impurities such as straw, wood, wire, earth, stones, etc. must not be attacked by enemies wax: mouse, moth, mildew. Poor remnants of wax crumbly when squeezed by hand, while rich in real lumps. Remnants of wax color close to the color of old comb with poor flavor on wax. If smells of solvents, it is not good. Humidity should not exceed 7%, which can be roughly determined. Wax content also determines the approximate organoleptic comparing the pattern and should not be below 25%. The larger quantities will be controlled laboratory in the prescribed manner. Wax pests: The biggest pests are wax or wax moth caterpillars and mice. Wax can be attacked, and one type of bacteria that feed on honeybee bread from the comb and damaged different mold. Wax moth larvae feed on wax and honey beebread from the comb making galleries in the comb. This pest causes heavy losses particularly in the reserve comb out bee society, the apiary weak and sick society by helping the spread of the disease called galeroza because the tracks full of bee venom. Larvae live 30 days later transformed into dolls, and after 14 days in the adult butterflies. In favorable conditions (20-30°C) complete cycle takes 44 days at a temperature of 20°C is 120 days at 10°C metamorphosis stops, while at 0°C larvae, dolls and insects die in 12 hours. Cold is the biggest opponent of moths. For this reason, some beekeepers in the fall, before discarding frames in reserve, put it in the freezer, those who have attacked moth. Protection against of this pest is disinfecting combs, and annual disinfection of all combs of reserves. Mice are rodents to 12cm long and can be a house, fields, and forest. These are very tough enemies of the bee, and transmitting the disease. Entering the hive in the cold season, in the fall or winter it attaches litter in hive and eats honeycomb with honeybee bread and honey, and bees. They bite the wood frames and hives dirty around the floor and on the frames. Their activity disturbs bees therefore consume more food and suffer from diarrhea. Honeycomb from mice

need to be replaced because it avoids and nuts. Good protection of mice combs are to be set up in October, and if there are too many mice to perform pest control in the apiary. Common vole shrewmouse is a dangerous pest in the apiary. It looks like a mouse but it is much smaller, has a small head so much easier to pass through the openings. While the mouse has a section head 10mm common vole has only 4mm lives in underground galleries, and most loves mountain forests, glades and is quite widespread. Her head looks like a mole's head with a pointed snout, ears and eyes are much smaller, and the tail is shorter. It is especially dangerous because while the mouse eats honeycomb she eats bees. Beekeepers should not be late in the fight against these pests because losses of them very large. Mycoses are parasitic diseases caused by various species of fungi. Some fungal infections caused by beekeepers as aspergiosis. In the corners of the hive are different mold spores, diseases caused by these fungi represent over 30% of bee diseases such as askoferoza (chalk brood) or aspergilioza (stone litter). Mold or fungi feed on organic matter from the comb or dead bees. Development degrades the environment in which they can cause a variety of diseases. Old comb with honeybee bread are suitable for the development of mold. These fungi produce toxins, some of which poison the bees, especially when adequate moisture and temperature hive. Mildew can be induced and inappropriate grazing in a zone, especially due to the high acidity of nectar. To avoid moldiness comb is necessary to ensure good ventilation, and warehouses where they stored spare comb, humidity should not be higher than that outside. Of course it is necessary annual disinfection.

#### Keeping honeycomb:

Well-organized apiary during the year must have a sufficient amount of frames with spare comb good quality. This means that the beekeeper must provide the appropriate conditions for the preservation of the honeycomb. Spare honeycomb kept indoors with the possible impact of appropriate insecticide. To do this, use cabinets, shelves or rooms that occasionally deration and disinfected. It should keep only good quality honeycomb while frames with brood (closed or open) right blend and bases disinfected bases need to be separately so that separate it from light brown, honeycomb with honey comb with powder. Honeycomb with honeybee bread should sprinkle with powdered sugar to avoid mold. Protect the room from the presence of mice and wax moth because they are very dangerous for spare cores. A space in which to keep spare cores should be closed, dry and in the summer it has a lower temperature. For this purpose the best basement dries rooms that are well sealed. The active season bases and extensions should be so spaced that provide airflow between the honeycomb and access vapor disinfectants. Disinfecting used sulfur, naphthalene, and more. The process consists of carbonation honeycomb sulfur dioxide resulting from the combustion of sulfur in the room. The dose that is used is 50g sulfur cubic meter warehouse space. Sulfur is burned at 120°C (in one court) blue flame producing sulfur dioxide. Gas kills caterpillars and butterflies wax moth and egg remains, and is therefore in the current season need to repeat the treatment after 10-15 days. Burning sulfur in the damp room creates acid H<sub>2</sub>SO<sub>2</sub> detrimental to all metal parts, especially the wire, but also acts as an insecticide because it can remain in the comb and so poison bees. For these reasons, we must before putting the combs in the hive to make sure ventilation and washing old comb. Honeycomb put at least 30min in the water after which is centrifuged and dried in the sun. Another procedure is set mothballs in areas where it is stored to the closet or warehouse. Naphthalene is white hydroscope powder that evaporates at room temperature and prevents the development of caterpillars. Get in honey (even when the lid), creating an unpleasant taste. Has great acidity and insert standards. Before re- starting the beehive honeycomb, which is treated mothballs, should aerate 2-3days. There are other methods of destruction and wax moths and microbiological using pathogenic microorganisms. Beekeeping practices has some interesting ways of

keeping the honeycomb out of enclosed space saving space, transport, ventilation, and washing. When hives follow with strong bee society honeycomb is left in installments under clew. Honeycomb is under constant protection of bees and implemented to better winter because the club has risen and less accessible to the cold winds. Some beekeepers kept extensions with honeycomb on apiary outside. Honeycomb is supposed to be light and no honey that would not have caused the bees. On the stand, sort 6-8 bodies with honeycomb, with the top and bottom ventilation over the grid. Body can plaster tape, if not sealed well. Old and invalid comb should not be kept for blends. If you cannot immediately blends honeycomb should be removed from the frames rid of wires and calendar strainer or manually in lumps that are easier to keep and fewer attacks moth.

Industrial processing of wax:

It is estimated that the processing of wax by beekeepers in ruins with remnants of wax remains 45% wax with the proper functioning and good equipment. Even if the beekeeper can allocate 75% of wax means that the rest is lost. There is an obvious need for the industrial processing of wax and buying remnants of wax. For industrial processing needs special installation of larger capacity. When pressing the warm are necessary hydraulic presses large capacity up to 140 atmospheres, heating and melting remnants of wax and crushed honeycomb is made in kettles with water that is heated to 120°C. Principle extraction is the same as in the apiary, but higher capacity. In the industrial production are used: Smelter wax sump for liquid wax applied extraction dissolving wax machine for industrial lifting quality wax, a device for the production of honeycomb, making installation of honeycomb casting, installation for making hourly basis through the creation of bands, lines processing wax honeycomb.

### **Conclusion**

Increased production of wax must be important for beekeepers and honey production. Security beeswax depends directly of the critical points of wax from: ways of obtaining's composition, processing, storage, transport, sales and so on. Wax is widely used in the world, even here in RS the demand for clean wax growing.

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## ECONOMIC ANALYSIS OF FEED INGREDIENTS IN DAIRY COW RATION

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### Abstract

Cow diet may largely affect the financial outcome of milk production. Ration formulation necessitates the satisfaction of nutrient requirements in terms of both animal metabolism and production-related needs to ensure expected milk yield per cow. Practice shows that feeding costs can be reduced if concentrate is replaced by good quality roughage whenever possible, with care taken to make sure that compound feeds have sufficient amounts of minerals and vitamins. Rations well-balanced in terms of the type, amount and price of the feed ingredients used can lead to positive economic performance in milk production.

The objective of this study was to analyse the effect of certain feed ingredients in dairy cow ration on the amount and price of milk produced. Research was conducted at a commercial dairy farm in Central Serbia. The test cows were fed two diets, one of which contained brewers' grains. The study involved economic analysis of ration per dairy cow, and monitoring of the average milk yield per cow and milk purchase price.

**Key words:** costs, cow diet, prices, brewers' grains.

### Introduction

Dairy cows diet necessitates properly balanced rations that contain all the necessary nutrients available in quantities that meet optimal cow diet requirements and ensure low ration cost. Research conducted so far suggests high feedstuff cost, accounting for some 60% in the total milk production. Rations can also be supplemented with food industry by-products with care taken to ensure that the utilization of some cheaper components in cow diet does not affect milk production response. In our studies, dairy cows diet rations involved brewers' grains and their effect on milk production response was monitored. Brewers' grains are by-products of brewery industry, and can be utilized in both wet and dried forms. The former is composed of malted barley and barley grain, and tends to spoil easily due to its 75–80% water content. It is classified as a protein feed. Some earlier studies and practical experience suggest its promoting effect on milk production response in cows. Recommended daily intake of brewers grains should be between 5–10 kg and should be fed after milking so as to ensure that milk does not take on its specific smell. It is believed that the introduction of brewers' grains in dairy cows diet increases milk yield and reduces its cost due to the greater amount of digestible proteins and crude fat per cow. The negative aspect of brewers' grains is the lack of tryptophan as well as the shortage of starch and sugar in its proteins, therefore high intake of brewers' grains (in excess of 15 kg) may have an adverse effect on digestibility. For this reason, brewers grains' are recommended to be supplemented with other feeds, such as roughage or some other by-products feeds, e.g. dry sugar beat pulp, apple pulp, etc. Aguilera-Soto et al. 2009 reported on no significant changes in pH rumen when feeding 15% brewers' grains. As recommended by Howard (1988), dried brewers grains' daily intake in dairy cows can vary from 2.7 to 4.54 kg per cow. West et al. (1994) report that wet brewers' grains can be used as a concentrate (up to 30%) or as a supplement to silage (Miyazawa et al. 2007, Polan et al. 1985). The latter also report on a positive impact of wet brewers' grains on both quantity and quality of the milk yielded.

In areas with no available silage and farms in close vicinity of breweries, brewers' grains are, along with hay and concentrates, the basic component in Total Mixture Rations (TMR) (Despotovi 2010). The largest quantities of brewers' grains are most commonly utilised as wet immediately upon

the receipt from breweries. Brewers' grains ferment rapidly and spoil easily due to the high moisture content, particularly in summer months. For a longer period of time, brewers' grains can be stored as silage (Dini and Oršević 2005). Regardless of the form it is used in, wet or dry brewers grains are an excellent replacement for concentrates and other components (corn and soybean) used for cow diet (Dhiman et al 2003, Firkins et al 2002). Care should be taken regarding the amount of brewers' grains to be used as a replacement in cow diet as it depends on specificities of feedstuff it replaces.

### Material and Methods

The research was conducted at a commercial dairy farm in Šumadija, Central Serbia. The testing involved 300 high yielding dairy cows of Simmental breed. A group of 175 cows in the different stages of lactation were selected for the study. The cows were fed two different diets over a particular period, and the quantity of milk produced and ratios of the obtained production results were monitored. The testing was performed at 10-day intervals, i.e. Dec.1–Dec.10 and Dec.15–Dec.25, 2012. Over the first 10 days, cows were fed TMR rations of compound feeds consisting of wet brewers' grains, alfalfa hay, straw, corn silage and concentrates. After a 10-day break, the same group of cows was fed identical diet, except for brewers' grains, which was excluded from the TMR rations. Care was taken to ensure well-balanced rations, in terms of the feed type, dairy cows needs and price of the feed ingredients. Dairy cows rations were made employing the linear programming method modified in Excel program. Additionally, milk yield and milk purchase price were monitored over the testing period.

### Results and Discussion

One of the concepts of providing rational dairy cow diet is based on maximum utilisation of good quality roughage which primarily includes silage. Basically, the TMR is made up of hay, silage, and a concentrate share which is balanced as needed throughout the year so as to ensure rations stability. Multiple feeds-based diet could provide higher-quality rations which are well-balanced in terms of the content of particular nutrients. It is desirable that high yielding dairy cows are fed rations composed of versatile roughage and concentrate feeds, as these can improve the volume and consumption of food, additionally exerting the positive effect on appetite in cows. TMR rations can also be supplemented with food-industry by-products when these are available. In our study we used wet brewers' grains. The initial objective of the study was to ensure constant milk yield and reduce feeding costs per cow. The feeding costs can be reduced if a portion of the concentrate is substituted with quality roughage which can be produced on-farm. In dry seasons with insufficient amount of hay the diet can also be supplemented with straw which is much cheaper and can serve as an alternative solution, as was the case in our study.

**Tab. 1. Cow diet with TMR rations involving brewers' grains**

Feed	Quantity kg	Price in euros	Total SM kg	Total energy. NEL	Total SSP in g	Ration price in euros
Alfalfa hay	0.4	0.2	0.34	1.572	56	0.08
Straw	2.0	0.045	1.7	6.94	20	0.09
Corn silage	30	0.04	8.4	46.8	360	1.2
Brewers' grains	6	0.1	1.38	7.44	264	0.6
Limestone	0.27	0.025	0.2673	0	0	0.00675
18% concentrate	7	0.4	6.16	33.25	840	2.8
Total			18.25	95.00	1540	4.78

Over 10-day period, a group of 175 dairy cows were fed TMR ration which involved wet brewers' grains, Tab. 1. The rations were distributed once a day, in the morning upon milking. It was done by using feed mixing wagon.

In our study, the TMR rations were based on corn silage produced on-farm, on both its own and leased land areas. Other feeds were purchased at market prices. Care was taken to balance rations, meeting the needs of cows, as well as reducing total feeding costs.

**Tab. 2. Cow diet with TMR rations without brewers' grains**

Feed	Quantity kg	Price in euros	Total SM kg	Total energ. NEL	Total SSP in g	Ration price in euros
Alfalfa hay	0.4	0.2	0.34	1.572	56	0.08
Straw	2.0	0.045	1.7	6.94	20	0.09
Corn silage	30	0.04	8.4	46.8	360	1.2
Limestone	0.27	0.025	0.2673	0	0	0.00675
18% concentrate	7	0.4	6.16	33.25	840	2.8
Total			16.87	88.56	1276	4.18

Tables 1 and 2 show TMR rations used in dairy cows diet. Whole grain silage supplemented with 7.0 kg concentrate was a major component of the diet. The rations contained only 0.4 kg hay (this was due to the high price of alfalfa hay which amounted up to 30 cents in the last year). Hay was replaced with 2.0 kg straw so as to ensure the cows' needs for cellulose. Over the first 10 days cow diet included 6 kg of wet brewers' grains, Tab. 1. After a 5-day break, which was in agreement with recommendations in literature that feeding cows with brewers' grains should not exceed 10–12 days, the study was continued over subsequent 10 days when rations did not involve brewers' grains, Tab. 2.

**Tab. 3. Comparative survey of yielded milk (in litres) over period of feeding with and without brewers' grains**

Num. of days	Period of feeding cows with brewers' grains			Period of feeding cows without brewers' grains		
	Num. of cows	Daily milk yield l	Average milk yield per cow l	Num. of cows	Daily milk yield l	Average milk yield per cow l
1	174	3069	17.64	174	2822	16.22
2	175	3199	18.28	173	2806	16.22
3	175	3086	17.63	174	2514	14.45
4	175	3065	17.51	174	2614	15.02
5	175	3097	17.70	176	2627	14.93
6	175	2974	16.99	176	2698	15.33
7	175	2927	16.73	177	2381	13.45
8	175	2770	15.83	175	2574	14.71
9	175	2825	16.14	175	2625	15.00
10	176	2781	15.80	175	2749	15.71
Average	175	/	17.02	174.9	/	15.10

Given the anatomy of the digestive tract of ruminants and the important role of symbiotic microflora of the rumen, it is of major importance that ration composition varies as little as possible, as otherwise it can have an adverse impact on both the efficiency of feeding milk yield (Jovanovi et al., 2000). If for some reason feeding cows necessitates any changes, it should be done gradually, and over a longer period of time (Grubi and Adamovi, 2003).

Groups of cows tested over both 10-day feeding periods involved averagely some 175 dairy cows. The data given in Table 3 infer that the average milk yield over the period of feeding without brewers' grains varied between 2,381 and 2,822 l milk, i.e. 15.10 l standard quality milk per cow, whereas the period of feeding cows with brewers' grains gave milk yield 2,770–3,199 l, i.e. 17.02 l per cow.

The utilisation of brewers' grains in feeding dairy cows averagely increased milk yield by some 1.92 l. Similar results were recorded by Palaševski et al. (2007) who reported on increase in milk yield by 1.5 l per cow among Holstein-Friesian breed.

**Tab.4. Value of milk yielded over the two feeding periods**

TMR ration	Average milk yield per cow (l)	Milk purchase price in euros	Milk value in euros
I*	17.02	0.4	6.81
II*	15.10	0.4	6.04

\*I period TMR ration with brewers' grains

\*II period TMR ration without brewers' grains

In the period of feeding dairy cows with brewers' grains, the average milk production per cow amounted to 17.02 l (Tabl. 4), milk value being 1,191.75 euros within the tested 175 cow-group. The results infer that milk value over the period of feeding cows with brewers' grains-supplemented rations was daily higher by 134.75 euros.

**Tab.5. Financial results of milk production as related to feeding costs**

TMR ration	Milk value (euro)	Feeding cost per cow (euro)	Difference in milk production on daily basis (euro)
I	6.81	4.78	2.02
II	6.04	4.18	1.86

The difference in milk production on daily basis over the feeding period which included brewers' grains amounted to 2.02 euros, i.e. 353.5 euros based on the tested group of cows (175), whereas the feeding period which did not involve brewers' grains gave 1.86 euros per cow (Tab. 5), i.e. 325.5 euros within respective group of dairy cows. The calculation points to some 28 euros daily difference between the two feeding modes, or 280 euros over the period of feeding cows with brewers' grains supplement. On annual basis, feeding dairy cows with brewers' grains-supplemented rations on averagely 200 days a year can give 5,600 euros, which is not a negligible amount for the business of any commercial dairy farm.

### Conclusion

The objective of our study was to show how diet components, roughage and brewers' grains in our study, may increase milk production and overall financial outcome accordingly, given that the utilisation of feeds above gave higher milk yield at a lower price. The examination conducted has confirmed that the brewers' grains in cow diet have a positive impact on milk yield and allow for some savings in feeding cows on large commercial farms.



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**SEROTYPE AND BIOTYPE PREVALENCE OF AVIAN PATHOGENIC  
ECHERICHIA COLI IN ALBANIAN POULTRY INDUSTRY**

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**Abstract**

A total of 104 *Escherichia coli* strains isolated from affected and apparently different poultry species, bred within the Albanian territory were serotyped and biotyped. Although *Escherichia coli* is normally present in the microflora of the poultry intestinal tract, certain subsets of *extraintestinal E. coli* strains termed as *avian pathogenic Escherichia coli* possess specific virulence factors that in previous studies have been associated with the avian colisepticaemia. Eight different serotypes were identified, where O86 resulted the most prevalent serogroup 15, 4%. Eleven biotypes were identified by the fermentation of five different sugar mediums by *E. coli* isolates. The most prevalent biotype was B31 (28, 84%). Various serobiotypes were identified, 98, 8% of *E. coli* strains were positive to rhamnose fermentation. This study objective was the comparison of the main characteristics of Avian Pathogenic

*Escherichia Coli* isolated strains with the Avian Faecal *Coli*, in order to ascertain whether Avian Pathogenic *Escherichia Coli* or Avian Faecal *Escherichia Coli* are distinct. The results obtained from the two groups reveal for major and fundamental difference which is reflected clinically.

**Key words:** Colisepticaemia, *E. coli*, APEC, Biotype, Serogrouping

**Introduction**

Colibacillosis is one of the principal causes of morbidity and mortality in avian species and is responsible for severe economic losses in the poultry industry (Dho-Moulin & Fairbrother, 1999). The disease in poultry is due to several virulence factors, which constitute various groups of similar *E. coli* strains differentiated by the specific pathotype (Kaper et al., 2004). Since *E. coli* infections in poultry may take any clinical form including colisepticaemia, peritonitis, cellulitis, salpingitis, synovitis, omphalitis, air sacculitis and coligranuloma (Barnes et al., 2003), it seems reasonable that multiple pathotypes of “disease-causing” avian *E. coli* might exist. However, these different forms of the disease are similar in their extraintestinal tropism. Previous studies have demonstrated that APEC possess virulence properties which promote the bacterial colonization and/or tissue invasion, subsequently leading to the colibacillosis development (Delicato et al., 2003; Dho-Moulin and Fairbrother 1999; Dozosis et al., 1992; Foley et al., 2002 and La Ragione et al., 2002). Never the less AFEC (Avian Faecal *Escherichia Coli*) strains are considered as normal part of intestinal microflora, certain *E. coli* strains are able to cause infection, and this ability due to the specific virulence genes designates them as APEC (Avian Pathogenic *Escherichia Coli*) (Dho-Moulin and Fairbrother, 1999). Several studies have revealed that generally many avian septicemic *E. coli* are grouped into a limited number of O serotypes as: O1, O2, O15, O35 and O78 (Cloud et al., 1985; Dozosis et al., 1992; Gross, 1991; White et al., 1998). During recent studies several isolates from affected poultry specimens have mainly belonged to O18, O81, O115, O116

and O132, which merges a signal of emergency for new pathogenic serotypes (Barnes et al., 2003). However, other studies have shown that a wide antigenic diversity exists among avian pathogenic *E.coli* strains (Allan et al., 1993; White et al., 1993) and the involvement of a particular O serotype in the infection appears to vary by the geographic region. Surveys on the virulence abilities of APEC strains have demonstrated genetic resemblances with human extraintestinal and uropathogenic *E.coli* strains (Kylie et al., 2005; Sorsa et al., 2001). Furthermore, several studies have shown that some APEC strains could belong to the same clones of human extraintestinal pathogenic *E.coli* (Achtman et al., 1986; White et al., 1993; White et al., 1990).

In this paper, we report the results of an epidemiological study on the circulating *E.coli* strains within the Albanian territory; comparing 74 *E.coli* isolated from colibacillosis affected poultry with 30 AFEC strains. For this aim, a total of 104 *E.coli* strains, from apparently healthy birds and colibacillosis affected ones were serotyped. More over the belonging biotype has been assessed.

### Materials and methods

#### Bacterial strains

A total of 104 *E.coli* strains, 74 from clinically confirmed cases of poultry colibacillosis and 30 from visceral organs of slaughtered apparently healthy birds were tested. The sampling was performed in broth rural and intensive breeding flocks, in different random geographical areas within the Albanian territory. Also different ages and poultry specimens were involved in the study.

Table 1. Avian *E.coli* isolates sampling distribution according to clinical signs, the breeding type and avian species

Avian species	Apparently healthy birds		Colibacillosis affected birds		Total of <i>E.coli</i> isolated strains
	Intensive Breeding	Rural Breeding	Intensive breeding	Rural Breeding	
Broilers	6	18	14	22	60
Layers	0	6	28	0	34
Turkeys	0	0	0	10	10
Total	6	24	42	32	104

Samples of fresh visceral organs from different poultry specimens with colibacillosis lesions (liver and spleen) and from apparently healthy birds were cultured on MacConkey agar (OXOID) and incubated at 37 ° C for 24h. The biochemical identification was performed using the API-20E system (Bio-MEIREUX). All the *E.coli* isolates were stored at -80 ° C in Brucella broth (OXOID) with 20% of glycerol till use.

### Fermentation of lactose

Test and control organisms were plated on MacConkey agar (OXOID) and incubated overnight at 37 °C. Isolates were considered positive to lactose fermentation if pink colonies were observed (Forbes et al., 1998).

### O-antigen serogrouping

The serotype of each isolate was determined using a slide agglutination technique on a panel of 40 different anti-O-sera, according to the laboratory methods and international literature of clinical diagnosis for poultry, rabbits and livestock (Blanco et al., 1998). A panel of 40 different specific antisera (O1, O2, O4, O6, O8, O9, O10, O11, O15, O18, O20, O21, O22, O26, O45, O49, O64, O73, O75, O78, O83, O85, O86, O88, O92, O101, O103, O109, O111, O128, O132, O138, O139, O141, O147, O149, O153, O157). A suspension of each isolate was diluted in 1 ml PBS and heated for 1h at 100 °C. A 5µl sample of each was mixed with quantity of an O-serotype antiserum. Agglutination on a glass slide within 1 min determined the respective serotype.

### Biotyping

Fermentation activity was tested on phenol red agar base, supplemented with 1% of each selected carbohydrate, in square Integrid Petri dishes (according to Difco Laboratories, Detroit, Mitch.). *E.coli* strains were inoculated as spots on the medium. The results were read after 24 and 48h of incubation at 37 °C. To assess the belonging biotype we have used the simple biotyping scheme of Camguilhem & Milton, 1989. The score number was assigned to every positive fermentation reaction as follows: D-raffinose (test score 4), L-rannose (test score 16), dulcitol (test score 2), sucrose (test score 8) and sorbose (test score 1).

## Results and discussion

Lactose fermentation - The abilities of APEC and AFEC strains to ferment lactose were determined by standard methods. Of the APEC isolates, 91% were positive to lactose fermentation; whereas 99% of AFEC isolates were lactose positive.

Serotyping – The serotyping was performed on a total of 104 avian *Escherichia coli* strains, from which only 44 isolates were typeable (42, 3%). Among the revealed serotypes were O2, O8, O15, O73, O86, O102, O115 and O139. The most frequent serotype was O86 (15, 4%) and the less prevalent were O15, O101 and O115 (1, 9% each). In medium level 4 other serotypes were detected: O2 (5, 8%), O8 (5, 8%), O73 (5, 8%) and O139 (3, 8%). The serotype O86 was more frequent in colibacillosis affected birds (87, 5%) comparing with 12, 5% of AFEC strains.

The serotypes O8, O15, O101 and O139 were only revealed from colibacillosis affected poultry, as the O115 belonged to isolates originated from apparently subjects. 60 of tested strains (57, 7%) did not belong to any serotype of the panel test, *as they were typeable with more than one specific serotype or not serotypeable at all.*

Table 2. Relationship between serotypes of *E.coli* strains isolated from affected and apparently healthy poultry.

<i>E.coli</i> isolates from colibacillosis affected poultry				<i>E.coli</i> isolates from apparently healthy poultry			
Serogroup	Broilers	Layers	Turkeys	Broilers	Layers	Turkeys	Total (%)
O2	0	4	0	0	2	0	6 (5, 8%)
	2	2	2	0	0	0	6 (5, 8%)
O8	0	0	2	0	0	0	2 (1, 9%)
	4	0	0	2	0	0	6 (5, 8%)
O15	10	2	2	2	0	0	16 (15, 8%)
	0	0	2	0	0	0	2 (1, 9%)
O73	0	0	0	2	0	0	2 (1, 9%)
	2	2	0	0	0	0	4 (3, 8%)
O86	18	18	2	18	4	0	60 (57, 7%)
O101							
O115							
O139							
NT							
Total	36	28	10	24	6	0	104 (100%)

Biotyping – A number of 11 different biotypes was revealed (B0, B16, B17, B18, B21, B22, B23, B28, B29, B30 and B31). 102 *E.coli* strains (98, 8%) were positive to ramnose fermentation (those with test score 16 and up). The majority (82, 67% or 86 of 104 strains) of avian *E.coli* strains were assigned to only four biotypes (B16, B28, B30 and B31). Most of the strains belonged respectively to biotype B30 (25, 0%) and B31 (28, 84%). These biotypes were more prevalent on the isolated originating from infected subjects (B31) or/ and exclusively detected in APEC strains (B30). The relevant results are presented in table number 5 and the co-relation between O-serotypes and biotypes is listed in the table number 4.

Table 3. Relationship between biotypes of *E.coli* strains isolated from isolated from affected and apparently healthy poultry.

<i>E. coli</i> isolates from colibacillosis affected poultry				<i>E. coli</i> isolates from apparently healthy poultry			
Biotype	Broilers	Layers	Turkeys	Broilers	Layers	Turkeys	Total (%)
B0	0	0	2	0	0	0	2 (1, 928%)
	6	4	0	10	0	0	20 (19, 23%)
B16	4	0	0	0	0	0	4 (3, 84%)
	0	0	0	2	0	0	2 (1, 92%)
B17	0	0	0	0	2	0	2 (1, 92%)
	0	0	0	0	2	0	2 (1, 92%)
B18	0	2	0	0	0	0	2 (1, 92%)
	2	2	4	2	0	0	2 (1, 92%)
B21	2	2	0	0	0	0	10 (9, 61%)
	12	12	2	0	0	0	4 (3, 84%)
B22	10	6	2	10	2	0	26 (25%)

B23					6	0	30 (28, 84%)
B28							
B29							
B30							
B31							
Total	36	28	10	24	6	0	104 (100%)

O: B – serobiotypes. The most prevalent serobiotypes detected among the 104 *E.coli* isolates, respectively to frequency were: O86: B31 (3 strains); O8: B16 (2 strains); O86: B16 (2 strains). O86: B28 (2 strains) and O139: B30 (2 strains)

Table4. Relationship between serogroups and biotypes of *E.coli* strains isolated from isolated from affected and apparently healthy poultry.

Serotypes	Biotypes
O2	B21 (1 strain) B23 (1 strain) B31 (1 strain)
O8	B29 (1 strain) B30 (2 strains)
O15	B28 (1 strain)
O73	B17 (1 strain) B18 (1 strain) B31 (1 strain)
O86	B16 (2 strains) B17 (1 strain) B28 (2 strains) B31 (3 strains)
O101	B0 (1 strain)
O115	B16 (1 strain)
O139	B30 (2 strains)
Non Typeable	B16 (7 strains) B22 (1 strain) B28 (2 strains) B29 (1 strain) B30 (9 strains) B31 (10 strains)

In Albania, as in other developing countries colibacillosis occurs frequently in both breeding types of the poultry industry. However the mortality varies from low to very high, depending on the characteristics of strains involved in the infection. Strains of low pathogenicity mostly cause problems in poultry farms of poor management and hygiene, which can be easily controlled by several hygienic measures and/or antibiotic treatment. On the other hand recently, antimicrobial treatment failures are very common due to the high pathogenicity of *E.coli* strains or to the antimicrobial resistance. The serogroups identified on 104 *Escherichia coli* isolates were O86 (15, 4%), O2 (5, 8%), O8 (5, 8%), O73 (5, 8%), O139 (3, 8%), O15 (1,9%), O101 (1, 9%) and O115 (1, 9%).

This result is very important for better understanding the profile of *E.coli* strains circulating now days in Albania. Also the serogroups identified exclusively in APEC strains, which are considered virulent were: O8, O15, O101 and O139. This result is consistent with other reports where these serogroups are commonly associated with avian colibacillosis and

confirms their role of potential pathogens in the extraintestinal infections of poultry (Dho-Moulin and Fairbrother, 1999; Ewers et al., 2004; Giovanardi et al., 2005 and La Ragione et al., 2002).

Very interesting was also the relationship of O86 with the respective Biotype, in *E.coli* strains. The biotype B31 was the most prevalent in the strains serogrouped in O86, followed by minor percentages of B16 and B28. These biotypes as presented in the table number 3 are mainly frequent in APEC strains. This induces the hypothesis that the O86 serogroup identified in an avian *E.coli* strain may be a sign of virulent abilities.

No previous study in Albania is performed before on the biotyping of avian pathogenic and fecal *Escherichia coli*. According to the now known pathogenicity of several serotypes on the poultry health and their relationship with the identified biotypes, we can make sense that B30 and B31 are the most prevalent biotypes in colibacillosis infection outbreak. But a conclusion on specific biotypes among pathogenic or fecal *E.coli* isolates cannot be framed as a large percentage of the identified *E.coli* biotypes coincide with the non typeable strains.

The presence of a large percentage of untypeable strains has been a common characteristic of many previous studies about the phenotypic characterization of *E.coli* strains, depending on the geographical region. A reason for 57, 7% of untypeable strains may be that the serotyping assay was performed in Italy, where the panel of 40 anti-O-sera could not possibly include the specific serogroups circulating in Albania.

### Conclusions

In conclusion, our results support that a wide serological diversity among avian pathogenic and fecal *Escherichia coli* strains exists, because of the opportunistic nature of this bacterial genus. Predisposing factors (mycoplasmal or viral infections, environmental conditions and poultry breeding type) could be responsible for this wide serological diversity. These data are the first report on the serological and biotyping profiles of avian *Escherichia coli* circulating in the now days, in Albania territory, and they provide a database on what is different and similar with other countries.

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**PRESERVATION AND CARE OF INDIGENOUS SJENICA CHEESE DURING  
RIPENING PROCESS**

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**Abstract**

Sjenica cheese is one of our best types of cheese from the group of white cheese in brine. It is produced by indigenous technology in the area of Sjenica-Pešter plateau. The process of production takes place at individual farms, among which larger manufacturers exist. The raw material for the production of cheese is fresh, whole sheep and cow milk, which process of making cheese starts immediately after milking, without thermal treatment. Studies were performed over a wide area of Sjenica-Pešter plateau, by a method of survey. The survey was conducted in forty households, and it included questions related to raw materials, the very method of making cheese and process of production. Special emphasis is given to the conditions of storage, ripening and to the system of preserving cheese. The results showed that microclimate conditions have an important role in the system of storage and preserving cheese in the places for storing and ripening. This is especially important during the summer months, when high temperatures can cause serious defects and deficiencies of cheese, such as increased acidity, porosity, poor structure and rheological properties of cheese. Sjenica cheese is kept in a salt-brine whey which is released by the cheese, or it can be added. Brine provides the anaerobic conditions of storage and ripening, and also serves as a preservative. Taking care of cheese is consisted of regular cleaning of cheese surface and the inner walls of tubs, control of levels of brine and regular replacement of the same. Critical period of preservation are the first 15-20 days after the process of production, and its washing and cleaning is done twice a week. If necessary, regular process and control of level of brine and its replacement, are done twice of week, too.

**Key words:**Sjenica cheese, storage, ripening care.

**Introduction**

Hilly area has remained the center of indigenous production and processing of milk into dairy products. A typical representative of this group of products is Sjenica cheese. It is produced

by indigenous technology on individual farms of Sjenica-Pešter plateau area, from raw whole cow, sheep and mixed (sheep and cow) milk that turns into cheese without thermal treatment (Savi , 2011).

Sjenica cheese belongs to a group of white cheese in brine. One of the characteristics of this group is the cheese storage and ripening in brine, which is used as a preservative, provides the anaerobic conditions of storage and ripening, giving specific lactic sour and salty taste and contributes to brittle structure of these kinds of cheese (Codex Stand. a2, 2000).

Storing and ripening in brine requires special care during ripening system that significantly depends on the microclimate conditions in the facilities, because the production, storage and ripening of cheese mainly occur in the same places (Macej etal.,2006)

Cheese ripening time is the longest and most important operation in the production of cheese. Desired sensory characteristics and quality of cheese under controlled conditions of temperature and relative humidity, are depending on the type of cheese and the ripening lasts to mature for a few months to two years (Wilkinson, 1990).

During cheese ripening, complex biochemical and physicochemical changes occur, starting even from the raw milk and addition of rennet starter cultures. These changes usually occur in proteins, milk sugar and milk fats. (Fenelon & Guinee 2000, Fox 1989, Fox & Cogan 1990).

During ripening process, complex biochemical and physical chemical changes do not only occur, but structural changes and the quality of transformation in zero curd cheese also occur. Actually, the grain structure, is eventually turning into a quality homogeneous mass, which has the taste, aroma and texture characteristic of a particular type of cheese (Jovanovi, 2001).

A number of authors (Beresford et al., 1998, Fox & Cogan, 2000, Ross et al., 2000) consider the ripening of cheese as essentially enzymatic process, and in the process of ripening the following agents participate: wide, native enzymes of milk, starter culture and other non-starter bacteria.

White brined cheese is produced from raw, thermally not treated milk. This means that a significant role in proteolytic processes during ripening process, has native milk proteinase. In addition, in the production of these types of cheese, starter culture are not used and besides rennet addition and native proteinase, enzymes indigenous micro flora play a significant role. (Bara et al., 2006).

According to (Maj et al., 2009), high quality of indigenous types of cheese is the result of specific indigenous technology and high-quality grass and healthy pastures that are at an altitude of over 1,000 m, and that dairy animals are fed by.

### **Material and methods**

Studies included in this paper were carried out over a wide area of Sjenica-Pešter plateau by a survey method. The survey was conducted in forty households, which are selected at random. Prior to execution of the survey, we had a detailed research, field visits and a selection of typical production site. The survey included questions related to raw materials, the process of cheese making, curd treatment (draining, pressing) and salting. Special emphasis is given to the conditions of storage, ripening, caring and keeping the cheese from the moment of production to the moment of sale. The survey, among other things, contained issues that are significant and have an important influence on the process of storage and the system of cheese care during ripening, such as the organization of processing milk into cheese, then a way of packaging and packaging and care procedures themselves during ripening and storage of cheese.

As these factors significantly affect the acidity of the cheese, in some household samples of ripened cheese were taken: four samples of cow, sheep and mixed (sheep + cow) cheese, by which the average values of titratable acidity are determined by the method of Ternar and acidity (pH).

### **Results and Discussion**

The quality and overall value, durability and sensory characteristics of cheese, among other factors, significantly influence the microclimate conditions and a system of good and complete care of cheese during storage and ripening. The research result give a complete

picture of the conditions of production, processing method, the interest in increasing production and improving production technology and perspective of development of the entire area.

The organization of production: Depending on the size of farms and their organizations, cheese production takes place in the village throughout the whole year, and in households that go to the mountain-huts, production has seasonal character (May to October). Processing milk into cheese, ripening and storage are performed in facilities specifically designed for it. Buildings in the village are usually made of hard material while in mountain, dairy huts are made of different cheaper materials. But the best microclimate conditions for storage and ripening in summer months, provides dairy-hut with walls of wicker that provide good air circulation-draft, and the floors are of soil that is moistured regularly. The roof is covered with PVC foil, above which is a thick layer of straw, and thus excellent insulation is achieved. In these objects, a temperature in summer hardly exceeds 16-18C which is great for ripening and storage of cheese. The results showed that in the majority of households, cheese production, storage and ripening are performed in the same building in uncontrolled conditions, actually the preservation and ripening in the production area.

Stacking and packaging: Cheese is stacked successively in rows of arrival immediately after preparation. Along with stacking, salting of cheese is done. It is salted with coarse sea salt, and a way of salting applied dry salting. When one row of cheese is stacked and wooden circle put, a less burdensome stone weighing 1-2 kg is put on. Procedure is repeated after each complex cheese party until the vat is filled. When the vat is filled, a wooden circle and higher load is put on, a stone of average weight 3-6 kg depending on the size of the vat. Beside this mode, manufacturers apply the other, and that is to put on the load only when the vat is full.

When stacking cheese in vats, normal procedure is releasing whey which is done before each new row of cheese, until the vat is filled. To do this effectively, at the bottom of the vat there is an opening which is closed by a wooden plug. Draining whey is done in two ways. The first way of discharge of whey is that when the cap opens, the whey is released periodically, and then opening is plugged again. Another way to release whey is that the opening at the bottom of the vat is opened for as long as the vat is full, and thus whey continuously discharges. When the vat is full, and the burden is placed on the cheese, the hole is closed with a plug.

Such a procedure of stacking cheese, affects the structure which is fragile and brittle and when removing cheese from the vats, it is very difficult to extract and separate it into slices. In fact, at the moment of stacking of fresh cheese, the cheese contains a lot of whey, and when cheese mass with high water content is stacked and then load, comes "coalescence of slices, that when removing ripened cheese can not separate but are broken." Besides to its structure, the whey drain is reflected on other properties of cheese, especially the taste. If the whey is not drained, cheese would have been even of higher acidity as the curd that the young cheese releases after stacking contains significant amounts of lactose, which would lead to a significant increase in the acidity of the cheese while turning into lactic acid. The results showed that in terms of reducing the acidity of cheese, a much better way is a constant whey release, because it is fresh and leaves vat without fermentation. As these factors significantly affect the acidity of the cheese, in Table 1 their average values in the mature kinds of cheese are given.

Table 1. Values of titratable active acidity in mature indigenous kinds of Sjenica cheese.

Indicators	Kinds of cheese			
		Cow cheese	Mixed cheese	Sheep cheese
Titratable acidity ( $^{\circ}$ T)	Min	197	191	196
	Max	220	224	246
	$\bar{X}(n=4)$	208	213	220
Active acidity (pH)	Min	3.80	3.83	4.43
	Max	4.94	5.71	5.36
	$\bar{X}(n=4)$	4.23	4.40	4.83

Based on the data in Table 1.it can be seen that the values of titratable acidity were high or low pH values, which is characteristic of this group of cheese.

**Maturation:** Maturation of Sjenica cheese is made in a salt-own cheese whey, which young soft cheese releases. Actually, during the production of cheese, curd processing is simple – which means curd is not cut but by procedure with it, is tended to keep as much water-whey in it. After stacking cheese in packaging and loading, the level of whey must be above the level of the cheese. If level of whey is lower, the whey must be poured and still be above the level of the cheese, by which anaerobic conditions of ripening are fully completed. Ripening process takes an average of twenty to forty days, although it is considered that the cheese is achieved commercial maturity after twenty days of ripening. Changes in cheese during ripening are the most intense in this period, and after that period, changes of the basic parameters of the chemical composition of cheese are less emphasized, so in the later period of ripening and storage, the cheese is conserved itself.

Sjenica cheese ripens and is kept in wooden vats as well as plastic buckets of different capacity. Savic, 2010., states that the wooden vat is original packaging for indigenous Sjenica cheese that is the best in terms of length of the quality of the cheese . However, market and consumers demands inevitably introduce other packaging, plastic of less volume.

**Care:** Caring of cheese is a very complex and sensitive issue. Care is especially important during the summer months in hot daytime temperatures. High temperatures can lead to serious flaws and shortcomings in cheese such as increased acidity of the curd, the occurrence of hole formation, poor structure and rheological properties of cheese.

Care contains cleaning of the surface of the whey and inner walls of vat from mold, regular control of whey level and pouring and changing.

Cleaning the surface of the cheese and brine from mold is performed in the following way: in fact, on the surface of the cheese is a circle and a stone whose diameter does not fully coincide with diameters of vats. Most of the mold is concentrated on the bottom area of a wooden circle, then on the bottom of the stone and on the inner wall of the vat, and a small amount remains in the cheese. Cheese surface and interior walls of vats are cleaned with pure cloth, a wooden circle and a stone are washed with clean water. Such care in summer periods is usually done twice a week, and in the autumn periods every 7-10 days. Besides washing the level of the brine is regularly controlled, which is added in need, so that its level is always

above the level of cheese. Change of brine depends on the storage conditions of the cheese. If the objects are exposed to draft (huts) and cool (hard material), brine does not change, or is changed once in period of June and October. More frequent replacement of brine is necessary during high summer temperatures and there is a risk of high acidity of the cheese, which is negatively reflected on the structure and rheological characteristics of the curd mass. As long as the brine is bright and airy, there is no danger for cheese. As soon as the brine is mixed up, and becomes viscous and drags into the long and thick filaments which are difficult to tear, such brine must be immediately changed.

The best quality of the cheese and its organoleptic characteristics are if brine is made from whey. However, in the absence of whey, that can happen during the autumn and winter months, brine can be made of the drinking water, but it is preferable to be a spring water, that is not chlorinated. Preparation of brine (presolac) is done by adding salt into whey or water, the water is boiled, cooled and so cooled pours into the cheese.

### **Conclusion**

Based on the results, and these facts we can conclude the following:

- Indigenous Sjenica cheese ripens and is kept in uncontrolled conditions, usually at the site of production and the care is very complex and sensitive issue.
- Micro-climatic conditions in the facilities are crucial for storage, ripening and care of Sjenica cheese, and hence on the quality and durability of cheese.
- Since the most intense and fastest changes occur during the first twenty days of ripening, so at that time a special regimen of care and maintenance of cheese is conducted. In the later period of ripening care system is easier, because the cheese is itself "conserved" and can be stored for a longer period (6-8 months).
- Regular care of Sjenica cheese involves cleaning the surface of the cheese mold, controlling the level of brine and its reconstitution and regular replacement of the same.
- Original packaging, the best for quality of indigenous Sjenica cheese are wooden vats. However, the market and consumer demand the need for smaller commercial quantities, and therefore plastic packaging of smaller volumes are increasingly used.

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## **6. RURAL DEVELOPMENT AND AGRO-ECONOMY**

**AN AGRO-ECONOMIC CHARACTERIZATION OF THE NIGER HOUSEHOLD:  
CASE OF TAHOUA**

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**Abstract**

Since the big drought of 1984, the department of Tahoua in the Republic of Niger is very vulnerable to structural and temporal disruptions. The food vulnerability is defined as “*the analysis of coping strategies and reactions faced with the structural or/and temporary shocks, if the coping strategies are not effectives, the people are in a temporary or structural situation of food vulnerability*” (Andres and Lebailly, 2011a; Andres, 2012). The environment is much degraded by the wind and water erosion. Despite many opportunities to develop the rural sector, the region of Tahoua has a structural problem because the income is not very high and the major part of the population is poor. This paper analyzes the characteristics of households in function of the food system. The target is to classify the population in relation to the food agricultural systems. A global description is defined and characterized for the agricultural system. The paper is based on a survey realized during 2010 and 2011. The investigators have interviewed 420 heads of household. The results are analyzed by Excel and SPSS software. The statistical analysis is a comparison of averages and the descriptive statistics. The results show a difference between the north (pastoral) and the south (agricultural) of the region of Tahoua, especially, for the production of cowpea, the number of tropical livestock units, the number of fields. Furthermore, the systemic view is very important to identify the specific constraints and opportunities of each food system.

**Key words:** Characterization, agro-economy, Niger, Tahoua

**Introduction**

Located in the Center of the Republic of Niger, the region of Tahoua is limited by the Mali in the Northwest, the region of Agadez in the North and east, the region of Maradi in the east, the Nigeria in the South, and the region of Tillaberi and Dosso in the west (Comité Régional de Révision de la Stratégie de Réduction de la Pauvreté, 2006). This region is characterized by a sahelian climate. The population is mostly rural. Indeed, during the census of population in 2001, the rural population was representing 90%. The population is mainly composed of Haoussa (78.2%) and Touareg in the North (17.5%) (Republic of Niger, 2001). Currently, the population of Tahoua of the census of 2012 is equal to 3,327,260. The annual growth rate of the population is equal to 3.4% (Republic of Niger, 2013). The major sectors of activities are the agriculture and the livestock. Furthermore, the principal constraints are the weak precipitation, the wind and water erosion, and the weak fertility of soils (FAO, 2003). The characteristics of the rural and agricultural population are defined by the agricultural and livestock census realized in 2008. This census has determined that the farmers of Tahoua are estimated at 1,863,601 and the distribution of this population is six members per household. The farming population represents 75 percent of the population. The major practice of the heads of household is the agropastoralism (90% of the households).



Finally, the mixed crops are the most important crops and the follow lands decrease and represent 35,273 hectares i.e. 4.4%. The area of mixed crop is estimated at 619,119 hectares against 126,054 hectares for the monoculture (Ministère du développement agricole et des ressources animales, 2008a).

The target demonstrates the characterization of the households in function of the food agricultural system. The target is realized by an analysis of some variables: socioeconomic (active members, level of schooling, marital status, etc.); agronomic (principal production, tropical livestock unit, area, number of fields, etc.). The first part of the results describes the situation of the households per food agricultural system. The second part realizes a comparison between the food agricultural system and a classification of households in function of the variables identified.

#### Material and method

The results are obtained by an investigation of 420 heads of households distributed in the departments of Tahoua (Tchintarabaden, Abalak, Tahoua, Illéla, Keita, Bouza, Birnin Konni, and Madaoua). The sample is chosen in function of the population census in 2001. The database has been constituted by “enumeration areas” of the population census (Andres and Lebailly, 2013). The “enumeration areas” are defined as a “geographical area of 200 households on average” (INS, 2007 in Andres and Lebailly, 2013). These investigations aren't about the nomad households but on the non-nomads. The results are obtained with SPSS and EXCEL. The food system is characterized by the following variables: Tropical Livestock Unit, the average production of mil, sorghum, cowpea between 2007 and 2009, the number of storehouses, the capacity of the storehouses, the number of fields and the area. This food system will be also described in function of the socioeconomic parameters (marital status, age, and number of members per household, education). This data is distributed in function of the food agricultural systems per department.

The agrohydrometeorological center AGRHYMET has determined the food agricultural systems per each department of Tahoua (Madaoua, Birnin Konni, Bouza, Illela, Keita, Tahoua, and Tchintarabaden). Currently, the department of Tchintarabaden assembles two departments: Abalak and Tchintarabaden. The food agricultural systems are calculated in function of the production of each department and the livestock. The food agricultural system is food-producing system if the agricultural area of food-producing products (cereals) is greater than 70%. The mixed system is described by the area of cowpea (less than 20%), the area of rice (less than 35%) and the food-producing products (less than 70%). The pastoral system is located at the northward of the isohyet 300 mm (CILSS, 2000).

#### Figure 6: The food agricultural system of the departments of Tahoua

Finally, we have realized a comparison of averages between the four systems. The comparison of averages is realized with the following variables: the area, the number of fields, the TLU, capacity of storehouse, number of storehouse, and average of millet, sorghum and cowpea production. The eight variables are compared in function of the four food systems about Tahoua. The equality of mean is realized if there is a normal distribution (Shapiro-Wilk test) and homogeneity of variance. The tests reject the hypothesis of normality and equality of variance. We have realized a non-parametric test with the Krustal-Wallis test.

### Results and discussion

The average of the age of the head of household is 51 years old but the heads of household located in the pastoral system have an age (46) lower than the rest of the other systems. The number of member per household varies between 8 for the mixed agropastoral system and 10 for the mixed agricultural system. A majority of the heads of household are male and for the pastoral system, the heads of households are only male. The Agropastoral food producing system presents the higher rate of the female heads of household, this percentage is equal to 19% (Table 1).

**Table 2: The characteristics of the heads of households per food system**

Food agricultural system	Age of heads of households	Number of members	Percentage of the gender of the heads of households	
			Male	Female
Mixed agriculture	53	10	88%	12%
Food producing agropastoralism	53	9	81%	19%
Mixed agropastoralism	50	8	83%	17%
Pastoralism	46	9	100%	0%

The marital status is mainly polygamous and monogamous. Indeed, the percentage of monogamous and polygamous is upper than 80 percent in each system. But the monogamous represents the largest part of this percentage. Finally, the level of education isn't different between the systems. But there is a difference of ten percent between the illiterate heads of households in pastoral (45%) and agricultural system (54%). Furthermore, many people haven't accessed the primary or secondary school and even less for the college.

The principal crop of the households in the region of Tahoua is the mixed crop but the agropastoral and pastoral system show a slight difference with agricultural systems. Indeed, the percentage of households practicing the pure crop is higher than the percentage of pastoral and agropastoral systems (Figure 2). Furthermore, the principal association is the millet-cowpea and the millet-sorghum-cowpea. This association is confirmed by the agricultural and livestock census (Ministère du développement agricole et des ressources animales, 2008a). The association of millet-sorghum-cowpea in the agricultural mixed system is more used in comparison with the associations of the other systems (millet-cowpea). The other systems present a high percentage for the association of millet and cowpea.

**Figure 7: The distribution of type of crop in each food system**

The Figure 3 shows the result of this test. The result demonstrates a difference between the four food systems. The differences are established between the number of storehouses; the capacity of storehouses, the number of TLU, the number of fields and the average of cowpea production. The equalities of average are proved between the area, the production of millet and sorghum (Figure 3).

**Figure 8: Results of the non-parametric Krustal Wallis Test.**

## Conclusion

Finally, the paper has demonstrated the difference between four systems present in the region of Tahoua. The heads of household are characterized by an average age of 51 years and there are on average 10 members per household. A majority of the heads is married and illiteracy is a big characteristic of the head of households in Tahoua. The capacity of production and storage is very different in function of the specificity of the system. For example, the production of cowpea decreases in function of a south-north gradient and the TLU increases in function of the same gradient (south-north). The systemic view is very important to identify the specific constraints and opportunities of each food system.

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**QUANTITATIVE AND QUALITATIVE EFFECTS OF PROTECTING  
TRADITIONAL AGRO-FOOD PRODUCTS BY GEOGRAPHICAL INDICATIONS**

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**Abstract**

Geographical Indications are protected names of products deriving from a limited geographic area, produced throughout history in a traditional unique way, by using local specific resources. They are much more than simple trademarks and today are considered as a model of rural areas development. The aim of this review paper is to emphasise the effects of agricultural products protection by Geographical Indications, in qualitative (*e.g.* improvement of rural population living quality) as in quantitative terms (*e.g.* added value that these products gain in agricultural market). Geographical Indications (GI) overcome the dimension of pure economic benefit for producers and expand the range of effects to consumers, rural areas, and to entire regions and countries. It is estimated that on global level more than 10,000 products are protected with GI. The sales value of GI products registered in EU-27 was estimated at €4.3 billion in 2010 at wholesale stage in the region of production. The whole value premium rate in EU-27 for GI products was estimated at 2.23, which means that GI products were sold 2.23 times as high as non-GI products. A growing body of evidence shows that typical and traditional products protection by the means of geographical indications has brought about multifaceted benefits to rural communities living in the areas of origin and contributed to improving their livelihoods and quality of life.

**Keywords:** Geographical Indications, sales value, premium rate

**Introduction**

The WTO 1994 Trade-Related Aspects of Intellectual Property Rights (TRIPS) Agreement states: “*Geographical indications ... identify a good as originating in the territory of a Member, or a region or locality in that territory, where a given quality, reputation or other characteristic of the good is essentially attributable to its geographical origin*” (art. 22.1) (Belletti *et al.*, 2011). According to the World Intellectual Property Organisation (WIPO, 2010), a geographical indication (GI) is a sign used on goods that have a specific geographical origin and possess qualities, reputation or characteristics that are essentially attributable to that origin. GIs are protected in accordance with international treaties and national laws under a wide range of concepts. When GIs are legally registered, they take such forms as Appellation of Origin (AO), Protected Designation of Origin (PDO) and Protected Geographical Indications (PGI), depending on the categories defined in the various countries and, as such, they become enforceable (Belletti *et al.*, 2011).

Products deriving from a limited geographical area and whose production contributes to the preservation of local knowledge and local specific resources, undoubtedly contribute to increasing the quality of life, of both producers and consumers, and in the broader sense of

entire communities. In this sense, introduction of an institutional system of protection of Geographical Indications (GI) and valorization of systems in which they exist contribute to faster and sustainable development of rural areas. GIs represent a significant potential for many regions, not only in terms of increased revenue, but also in cultural and environmental terms (Bramley, 2011).

It is estimated that on global level more than 10,000 products are protected with GI. About 90% of these products are from the Organization for Economic Cooperation and Development (OECD) countries, while the remaining more than 160 countries have a few protected products (European Commission, 2008a). A much greater number of products are eligible to bear GI but they have no legal protection. However, not all GI products are equally successful in the world market. In many cases, indications of geographical origin are protected in one country but not in another or the purpose and form of protection often differ from country to country. For example, Feta and Champagne are protected in the EU but not in U.S. where the words “feta” and “champagne” have generic meanings.

This paper brings out some quantitative and qualitative data regarding the effects of protecting products originating from limited geographic areas with the use of specific local resources and traditional production methods.

### **Material and methods**

Analysing geographical indications for their economic, social and ecological impacts implies facing with two kinds of challenges. Firstly, it is difficult to obtain suitable data; and secondly, clear methodological parameters are necessary for the analysis of the data collected (Belletti *et al.*, 2011).

Sources of data used in this study were, in addition to the academic literature, the European Commission evaluation studies on GI. The first Evaluation of the Common Agricultural Policy (CAP) policy on Protected Designations of Origin (PDO) and the Protected Geographical Indications (PGI) was carried out in 2008 and the second one on the value of production of agricultural products and foodstuffs, wines, aromatized wines and spirits protected by geographical indications was carried out in 2012.

Results are presented in quantitative and qualitative forms. With quantitative indicators are presented: (1) the total sales value of products under GI, (2) the share of sales value of products under GI in total sales value of foodstuff and drinks sector, and (3) the ratio of market prices of GI products compared to products from the same category without GI. Qualitative indicators refer to the social and geographic parameters on whose development the protection of GI has effects. Quantitative indicators have a more operational character and their use value is higher for administrative purposes, while the qualitative indicators are of more theoretical nature and their use value is higher for academic purposes.

In this review paper there is no distinction between the effects of the GI system (supply chain and network), the protection schemes (legal framework) or cooperation projects or programmes aiming at implementing GI regulations and they are considered altogether (cf. Belletti *et al.*, 2011).

### **Results and discussion**

#### *3.1. Effects of geographical indication protection*

Numerous studies conducted in developed and in developing countries, clearly indicate a positive correlation between the protection of local products by GI and local community development. Among positive effects of the protection with GI is certainly necessary to enumerate the following (O'Connor and Company, 2005):

- Increased production, creation of new jobs and prevention of further displacement of population in rural areas;
- Achieving higher sales prices for products due to production methods and known origin;
- Increased recognition of areas of production;
- Achieving other indirect positive effects *e.g.* through the development of rural tourism;
- Contribution to the preservation of biodiversity, local knowledge and natural resources;
- Avoiding standardization of food production;
- Enabling offers of completely different and unique products.

GI products, besides the above mentioned effects on rural community development, also have positive effects on social features of communities from which they derive by contributing to social cohesion by making producers relying on each other, playing a positive role in lifting up local and national identity, and making producers and consumers proud of their unique traditional products (reference?). GIs production often has the potential to obtain positive environmental and social side effects (Belletti *et al.*, 2011).

Radman (2006), states that the geographical origin is a vehicle to increase the quality of food products for the following reasons:

- Use of GI contributes to a better organization of rural areas and population that perform a given production. Production and processing of agricultural food products according to the strict rules of traditional production contributes to retaining skilled people in rural areas;
- GI are themselves holders of greater value, because the ownership is of all inhabitants of the territory, and cannot be privatized;
- GI provide a more equitable distribution of added value along the supply chain, from production, over distribution to consumption;
- GI stimulate production of quality products, which results in greater competition in the market.
- GI contribute to the preservation of identity and cultural heritage of the production region. Being present on global market such products contribute to a positive image of the area of origin, and they encourage and enable producers to be more present on the international scene.

### 3.2. Economic effects of geographical indications

The sales value of GI products registered in EU-27 was estimated at €4.3 billion in 2010 at wholesale stage in the region of production, of which 56% is related to wine, 29% to agricultural and food products, 15% to alcohol and 0.1% to flavored wine. The sales value increased by 12% in the period 2005-2010. The greatest increase in sales during this period was recorded in the agro-food sector (+19%), mainly as a result of the registration of new GI. In the wine sector, the value of sales rose by 9% mainly as a result of increased selling prices (+8%), while sales volume increased slightly (+1%). France is the leading country with a total sales value of €20.9 billion, which makes 29% of total sales value of EU-27 (Chever *et al.*, 2012).

The total sales value of food and drink sector in the EU-27 in 2010 was estimated at € 956 billion. The share of GI was 5.7% in 2010. In the period 2005 - 2010 growth rate of the total sales value was +13.9%, while the growth rate of GI was slightly less (+12.1%). The largest share of GI sector was recorded in France (14.5% of the total food and drink sector), the three Mediterranean countries (Italy, Greece and Portugal), with approximately 10%, and Slovenia (6%) (Chever *et al.*, 2012). In southern Europe, the culture of protecting GIs is historically embedded. For instance, France has a century of history in promoting official origin-based quality signs (Sylvander *et al.*, 2007).

Economic data from developing countries is very difficult to find, however, there are some estimates. According to Giovannucci *et al.* (2009), for example, the total value of exports of Basmati rice from India in 2007 amounted to \$1.5 billion, while data from Pakistan from 2001 talk about the value of exports of \$250 million. Export value of Tequila from Mexico in 2007 was estimated at \$725 million. Total exports of Mountain green coffee from Jamaica brought \$24 million in revenue in 2008. By improving market access, GIs can lead to higher incomes through increasing the volume of goods sold. But GIs have a further potential income effect through the collective process of value creation (Barjolle and Sylvander, 2000) that could lead to the capturing of a premium. This GI embedded value is a mixture of economic, cultural and social values which derive from locality; the so-called “*immaterial dimension of food consumption*” (Reviron *et al.*, 2009).

Many studies provide evidence of price premiums for GIs from developed countries. These studies include willingness to pay surveys which have found that 43% of consumers in the EU are willing to pay a 10% premium for a product with GI labelling, while 8% of EU consumers have indicated a willingness to pay a 20% premium (Berenguer, 2004).

The whole value premium rate in EU-27 for GI products was estimated at 2.23, which means that GI products were sold 2.23 times as high as the same non-GI products. A comparison of the average value premium rates between schemes shows that GI wines are sold at a price greater than 2.75 with respect to wines without GI, GI spirits at a price 2.57 times higher compared to drinks without GI, and the lowest price premium rate, 1.55 times or less, is that recorded in the case of agricultural food products with GI.

The total value premium of EU-27 GIs was estimated at €9.8 billion. Wines contributed 65% (€9.3 billion), agricultural products and foodstuffs 19% (€5.6 billion) and spirits 16% (€4.9 billion).

Ex Yugoslavian countries are in a very different steps regarding its approach to the protection of local products by a GIs. Slovenia, who is full EU member since 2004, has 16 products bearing GI signs (European Commission, 2013). Croatia has entered EU with 13 products protected by GI on national level and none of them is protected on EU level (MPRH, 2013). Bosnia and Herzegovina still has no resolved conflict of jurisdiction which institution is responsible for registration of GI.

The economic benefits of geographical indications come mainly from differentiation: a special quality linked to the territory is acknowledged by consumers in the country and outside (Belletti *et al.*, 2011). In fact, the demand for GIs is based on the economics of product differentiation (Moschini *et al.*, 2008) so it affords producers a valuable opportunity for the creation of territorially-differentiated niche markets.

### 3.3. General effects of geographical indications

GI overcome the dimension of pure economic benefit for producers and expands the range of effects on consumers, rural areas, and to entire regions and countries. Bramley (2011) in a literature review on the socio-economic impacts of GIs dealt with the following issues: quality signalling in support of consumer and producer welfare; improved market access through differentiation and value creation; rural development dynamics; the preservation of traditional knowledge; and the preservation of biodiversity.

It is widely articulated that protected GIs may contribute to rural development (cf. Van de Kop *et al.*, 2006). GIs potentially impact rural development in two ways. Firstly, through the remuneration of specific assets directly involved in the production process. Secondly, the GI qualification process itself, by defining product standards and signalling territorial values embedded in the GI product increases the ability of capturing price premiums. Also, by preventing the diversion of income from misappropriation, GIs enable producers to



potentially enjoy larger income flows from their origin based production processes (Zografos, 2008).

GIs have for many years been the main pillar of the European Union's agricultural product quality policy and are seen as a strong development tool for lagging rural economies. The EU's perspective on GIs has been described as "*a legal and commercial basis for development of rural areas, the preservation of cultural heritage and the promotion of small and medium firms in the rural economies context*" (Sylvander and Allaire, 2008 in Hughes, 2009).

From the perspective of consumers, GI indicate the important characteristics of products that are not often checkable by a simple inspection control. For example, consumers cannot easily determine wine quality, nor its production process, or whether a cheese is made using traditional production methods. Geographical origin is indicated not only by the link between a product and the region of production, but automatically includes the application of a particular method of production, well-known characteristics and quality of a given production region.

A consumer's survey in the USA in 2005 has shown that 72% of respondents think that the geographical features of the place of production, such as land, affect the quality and taste of food (De Carlo *et al.*, 2005). Research carried out in 1999 in the EU, which was conducted on a sample of 20.000 consumers, showed that the main motive for buying a product of known geographical origin for 37% of respondents is the guarantee of origin of the product, 35% expected a better quality, 31% for specific place and method of production, and 16% for tradition (Berenguer, 2004). It further showed that 50% of respondents are willing to pay between 10% and 20% higher prices for products with protected geographical indications.

For rural regions GI can be a platform for affirmation and validation of unique socio-cultural and agro-ecological characteristics. They also have a positive impact on building the reputation of the territory, increasing sales of other products and strengthening rural tourism. In recent years, rural development policy strategies are increasingly based on strengthening local produce and other local values of the territory.

Certain regions and countries have a very positive experience in increasing income of farmers, processors and distributors thanks to products protected with geographical indications. Among such products that enjoy a reputation on the global level can be cited: French Cognac, Italian cheese Parmigiano - Reggiano, Jamaican blue mountain coffee, Scotch whiskey, citrus from Florida; all of which are protected with GI. It is well known that regions from which these products originate have achieved significant levels of economic development, which can be explained by adequate use of natural and cultural resources. Currently, 167 countries, mostly economically developed countries, have developed legal basis for the protection of geographical indications. According to the EC, the strategy for protection of GI has made the expected economic effects. Developing systems of protection of geographical indications is still a new area but certainly promising especially for countries whose agriculture is based on small family farms with extensive and traditional production systems. In fact, more and more cooperation programmes regarding GI framework and the registration of products are being launched in transition and developing countries (Barjolle and Salvadori, 2010).

Nevertheless, GI laws cannot by themselves guarantee benefits for rural development (Hughes, 2009; Belletti *et al.*, 2011). GI registration does not guarantee a fair distribution of value to producers nor positive environmental and social effects. These effects depend strongly on the quality of the supply chain governance and on the elements of the code of practices (Hughes, 2009).

## Conclusions

Traditional and typical products are the result of complex production systems strictly interrelated with many dimensions of territorial development going beyond economic issues to involve social and environmental dimensions as well. Geographical indications in their simplest form are signs that recognise the link between a product reputation, quality or some other characteristic and its geographical origin. It is undisputed that there are significant benefits attached to GIs. Geographical indications have positive effects on producers and the wider regions of origin. One can even speak about their positive impacts at the national level in some countries. The legal protection of GI products is often conceived as a lever for activating local development dynamics and defending territorial production systems with a high degree of site specificity against pressures coming from globalisation. However, achieving these dynamics is not a simple process and requires concerted efforts.

While the share of the value of sales of GI products in the total value of sales in certain countries reached 14%, there is nowadays in Bosnia and Herzegovina a general confusion in terms of laws and regulations, and the institutions responsible for the management issues related to geographical indications. Although Bosnia and Herzegovina under the Stabilisation and Association Agreement (European Commission 2008b) engaged to align this subject area with the current European legislation no significant progress has been recorded so far.

There are particular challenges to harnessing the potential socio-economic benefits of geographical indications. In addition to suitable legal protection, the GI process needs the support of appropriate institutions and policies. GIs are multi-dimensional instruments so informed policy decisions around GIs should therefore beyond economic considerations take into account the potential social and environmental benefits.

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## GOVERNANCE OF AGRICULTURE AND RURAL DEVELOPMENT IN EGYPT

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### Abstract

Agriculture is a major economic issue in Egypt, although its contribution to the GDP is gradually diminishing. It is evident that rural and agriculture growth is the key element towards reducing poverty, achieving food security and increasing demands for labor.

Egypt is facing great challenges regarding its economy in general, and agriculture and rural development (ARD) in particular, such as food shortage, water insufficiency for agricultural purposes, the extreme poverty among small-scale farmers, lack of coordination and integration between various stakeholders, complicated local administration system and centralization, and declined governmental investments in the agriculture sector.

Though, all these challenges require restructuring Egypt's governance in agriculture and rural development and institutional reform.

Therefore the current study aims at identifying agriculture and rural development policies and strategies in Egypt, public stakeholders involved in designing, implementing and evaluating ARD, besides analyzing the relationships and linkages between these actors. Additionally the paper provides an overview of the main international organizations dealing with ARD (*e.g.* UNDP, JICA, and FAO) and implemented projects.

Various analytical tools were used in order to give comprehensive overview about ARD in Egypt, including quantitative and qualitative methods. SWOT analysis was used to verify the gaps in the current flow of ARD strategy.

ARD strategy in Egypt needs to be re-structured and based upon coordination and integration between various sectors and stakeholders either national or international to avoid duplication and fulfill ARD goals and objectives that will eventually lead to "true" development, food security and poverty alleviation.

**Keywords:** Agriculture, Rural Development, Governance, Egypt.

### Introduction

The Egyptian administrative system was developed in 1960; it was an organizational technique by which the country's regions were divided into local units that practice their authority according to the constitution within their regional jurisdictions. The local administrative systems in Egypt include three main institutions: the high council of local administration, economic territories, and local units.

The local units include four levels: governorate, district (markaz), village and satellites. The village local unit acts within the public plan and manages all public facilities within its jurisdiction.

Agriculture in Egypt has witnessed significant developments over the last two decades with direct effects on the role of the agricultural sector in national income formation and exports

promotion. Such developments have also affected farmers' delivery as related to the cropping structure, applied technology, levels of income, and farmers' response to market changes. The share of agriculture sector in GDP is about 14.5% in 2011 (CAPMAS, 2006). During the last three years, growth rates improved, reaching 3.3% in 2006/2007 and declined to 2.7% in 2011 (MALR, 2009).

Egypt's population size reached 72,798,031 of which 31,370,925 inhabit urban areas representing about 43.09% and about 41,427,106 inhabit rural areas representing about 56.90% (CAPMAS, 2006). The agricultural labor force is the highest as compared with other sectors; it represents about 26.01% of total labor force, agricultural male labor represents about 29.08% and 9.67% are female of total labor force.

Rural development experiences in Egypt faced problems that chained its progress, and all lacked coordination and integration, financial problems, political conditions, poor management and most important factor, except "Shorouk" program did not reflect people's priorities and needs, though "good governance" or "rationale governance" could be the key solutions to overcome these problems.

Governance basically emerged within the developmental context of international organizations *e.g.* UNDP, IMF in 1989 as other stakeholder become partners in the developmental processes *e.g.* private sector and civil society organizations though it was necessary to achieve coordination and integration among these partners from one hand, and to improve "governance" within its structure in order to guarantee the success of any development exerted efforts. Governance became not only the main goal but a prerequisite for aid allocation to developing countries, which represents a question mark about the real purpose of this "buzz word" and its impact on the socio-political stability in developing countries.

The concept tackled different angles which mainly reflect these organizations agenda, but all shared four main features; first improving public management or improving governmental bodies' performance, second transparency, third accountability and forth is the rule of law. Some organizations widened their scope to include military expenditures and controlling corruption.

The IMF determined three dimensions for governance: the political system form, and the means by which power is exercised in managing the social and economic resources for development and finally the government (state) capabilities to design, formulate and implement policies and distribution of tasks (Al-Baradei, 2003). Though, governance concerns the state's ability to serve the citizens, it refers to rules, processes and behavior by which interests are articulated, resources are managed and power is exercised in society (The European Commission, 2008).

There are eight key principles grouped under three main themes for effective rural governance (Stark, 2005):

- Collaboration: crossing sectors (public, private, and non-profit), and crossing political boundaries and recognizing regions.
- Sustained citizen engagement: welcoming new voices (especially underrepresented individuals and youth) and visioning a different future (bottom-up process).
- Leveraging: analyzing a region's competitive advantages (focuses on strengths and identifies clusters), and strengthening competencies of local elected officials and engaging key intermediaries, besides investing local capital. Governance of a sector, and the way in which politics and institutions interact within that sector, will in practice have a critical impact on sector policies and services.

Governance assessments have traditionally focused on formal governance structures and processes and less on the interaction between actors and institutions. Recently, newer generations of governance analysis frameworks have emerged which adopt a more realistic and non-normative approach, and as such are more in line with political economy frameworks and approaches (DFID, 2009).

In light of the previous review and analysis, an important key question emerges regarding the current study dilemma; are there any rationale management and smooth flow of the decision-making processes that helps in solving or avoiding some or all threats and weaknesses in Egypt ARD? Is there any kind of cooperation and coordination among various actors? What about major problems these actors face and to what extent they manage their projects at operational levels? Are ARD programs implemented by these actors reflecting people's needs and priorities? And overall is there any RD strategy in Egypt?

All these questions reflect the current study's problem and in same time its objectives, as they all combined emphasis the important relationship between governance and achieving successful ARD policy that guarantees its sustainability.

The current study main objective is to identify whether good and effective governance “really” takes place in Egypt ARD or not. Therefore the following specific objectives were formed:

1. Identifying agriculture and rural development policies and strategies in Egypt;
2. Identifying public stakeholders (actors) involved in designing, implementing and evaluating ARD;
3. Analyzing the relationships and linkages between these actors.
4. Providing an overview of the main international organizations dealing with ARD and implemented projects.

### **Material and Methods**

The work is based on an extended secondary data review. The main sources were some Egyptian ministries (*e.g.* Ministry of Agriculture and Land Reclamation, Ministry of Economic Development) as well as some international organizations (*e.g.* FAO, JICA). The approach adopted is at the same time descriptive and explorative. Various analytical tools were used in order to give comprehensive overview about ARD in Egypt, including quantitative and qualitative methods. SWOT analysis was used to verify the gaps in the current flow of ARD strategy.

### **Results and discussion**

Egypt's agriculture sector faces great challenges, through analyzing its status as indicated in table 2, there are problems that affect the performance of this sector and all those involved in it, institutions, organizations or unions. Basically, there is a kind of separation between agriculture development and rural development, the three agricultural strategies since the 80's then 90's and the current strategy developed in 2009 focused mainly on agricultural development and neglecting to some extent rural development until the last strategy which allocated part of it to improving rural people's life and infrastructure and paid attention to improving the human capital via education, health, and socio-economic environment.

**Table 2: SWOT analysis of agriculture and rural conditions in Egypt**

<b>Strengths</b>	<b>Weaknesses</b>
<ul style="list-style-type: none"> <li>• Availability of labor force</li> <li>• Increased productivity in many crops</li> <li>• Improved export capabilities</li> <li>• Food gap reduced in some crops</li> <li>• Reformation of owner-tenants relationship</li> <li>• Existence of legislations and regulations</li> <li>• Various entities participate in ARD</li> <li>• Diversified production activities</li> </ul>	<ul style="list-style-type: none"> <li>• Lack of a rural development strategy</li> <li>• Unskilled labor</li> <li>• Fragmented agricultural lands</li> <li>• Youth migration</li> <li>• Water resource problems</li> <li>• High poverty and illiteracy rates</li> <li>• Lack of coordination among ARD stakeholders</li> <li>• Weak role of agriculture extension and cooperatives</li> </ul>
<b>Opportunities</b>	<b>Threats</b>
<ul style="list-style-type: none"> <li>• Establishing rural development strategy</li> <li>• Incentives to attract rural youth</li> <li>• Improved coordination and cooperation among relevant stakeholders</li> <li>• Enhancing SME's among rural youth</li> <li>• Training for unskilled labor</li> <li>• Improved rural finance and credit</li> <li>• Improving food exports</li> </ul>	<ul style="list-style-type: none"> <li>• Increased population rates and density</li> <li>• Losses in agricultural lands</li> <li>• Deterioration in soils</li> <li>• Political instability</li> <li>• Water scarcity</li> <li>• Absence of valuing agriculture lands</li> <li>• Deterioration in natural resources</li> <li>• Increased food demand and food import</li> </ul>

The sixth five - year plan for social and economic development (2007/2008-2011/2012) indicated that about 1392145 thousand Egyptian Pounds (EGP) is allocated for implementation of programs and projects by the Ministry of Agriculture and Land Reclamation (MALR), representing about 0.87% of the total budget allocated for social and development projects (Ministry of Economic Development, 2007a). Additionally, the second part shows that about 1105300 thousand EGP are allocated for MALR for implementing its economic activities *e.g.* agriculture, irrigation, land reclamation, financial intermediation, insurance and social security (Ministry of Economic Development, 2007b).

The targeted investments for agriculture and irrigation in 2012/2013 is about 12.4 billion EGP with an increase of about 141% of the previous year, which was about 5.14 billion EGP, due to doubled investments in private sector, which was about 7.4 billion EGP.

Rural development programs witnessed historically five main phases from (1882-1994), each phase was distinguished with specific features since the British occupation who paid attention only to cotton production, to the establishment of the Agriculture Credit Bank in 1931 to finance farmers, cooperatives and rural development efforts. Also community groups were formed to improve the living conditions especially among farmers. Then the Ministry of Social Affairs with a special division for farmers, and rural social centers were established in 1941/1942. In 1942 a law concerned with rural health was issued which allowed the government to allocate part of its budget for rural health programs and to establish agricultural units that provides extension services to farmers. Prior to the world war II the High Committee for Poverty Alleviation was established and provided an eight year plan that

coordinate among provided services, aiming at establishing a social centre in each village that includes a clinic, a rural school and a rural industrial training centre.

After the 1952 revolution the agriculture reform law was issued determining the size of land ownership, followed by establishing the General Services Council in 1953 to support the service programs particularly in rural areas. In addition to launching the combined units that provide various services, economic, social, health and educational to rural areas in 1956, in same year the unified cooperative law was issued.

The first five year plan took place in 1960/1965 and the state adopted the central planning policy during 1960-1973. The government paid attention to educational and health services mainly in rural areas, with no attention to infrastructure services. In 1960 the local administrative law was issued which enabled for the first time people's participation in designing projects and programs in rural areas and to some extent in its implementation.

In 1973 the Organization for Reconstruction and Development of the Egyptian Village (ORDEV) was established to be responsible about forming the executive plan, monitoring and evaluation. In 1976 the role of agricultural cooperatives was weakened due to the establishment of village banks that took most of their activities. In the 80's various projects and programs funded mostly by foreign institutions and agencies were implemented aiming at achieving economic and social changes in rural Egypt, but they lacked coordination and integration from one hand and overall development philosophy from another hand.

The five previously mentioned RD stages in Egypt basically stopped at 1994, yet the five year plan for social and economic development continued, but a sixth stage or phase could be added which took place in the period 1994-2017 as it includes a very recent integrated RD program, the National Integrated Rural Development Program "Shorouk" *i.e.* sun rise. The program was applied through ORDEV, which adopted a specific strategy that incubates all public and governmental efforts to achieve real rural development that assures integration and coordination and people's participation in the development process. Its philosophy depends on people participation is the core of development and the governmental efforts (financial and technical support) are complementary. The program was supposed to end in 2017, but could not due to financial and political issues. This program, to some extent applied governance technique when providing its institutional structure and technical support (Moharam, 2005) in order to assure smooth flow in all the developmental process and to guarantee coordination and integration among various actors and full participation either from people or civil society organizations.

There are various international organizations *e.g.* UN organizations, UNDP, FAO, JICA and national organizations *e.g.* ministries, Universities, research centers, private sector and extension all dealing with development in general and ARD in particular. Basically, most of these organizations deal with the notion of development that could be agricultural or small-scale enterprises or loan providers or charities. The governmental organizations particularly ministries provide development either in rural or urban areas, with few exceptions such as The Ministry of Agriculture and the Ministry of Local Development (MLD). At the government level, particularly in MALR, the direct provided ARD strategy is the Sustainable Agricultural Development Strategy (SADs), in addition to the other socio-economic plan implemented by other national actors. SADs vision is to achieve a comprehensive economic and social development based on dynamic agriculture sector capable of sustained and rapid growth, while paying special attention to underprivileged social groups and reducing poverty. Whereas its mission is to modernize agriculture based upon achieving food security and improving rural people's livelihood, through efficient use of development resources,



utilization of geopolitical and environmental advantages, and comparative advantages of different agro-ecological regions.

The strategy provides the following implementation mechanisms: institutional reform for agriculture sector and civil society organizations, developing different agricultural policies, development of programs and projects *e.g.* the national program to rationalize and upgrade efficiency of water use in agriculture, national program to develop field crops, national program for socio-economic development in rural areas (MALR, 2009).

The following represent examples of some major programs and projects implemented by some international organizations in Egypt. FAO projects in Egypt (1978-2010) include: Egypt-Al-Beheira rural development, irrigation improvement program, assistance in agricultural policy analysis, promotion of agribusiness investment by private sector, strengthening the nation agricultural extension system, assisting small-scale poor fishermen to increase their fish catch. In general FAO implemented about 150 projects in Egypt within the same period that amounted US\$ 55,234,702 (FAO, 2011). As for JICA, the following represent major projects implemented in Egypt: introduction of clean energy by solar generation system and drainage water quality control for irrigation in Middle Delta (JICA, 2013).

The UNDP continue to focus on poverty reduction via inclusive growth and job creation through the policy environment and support services for small and medium enterprises and facilitating their access to credit. Also better targeting the poor by enhancing poverty monitoring and promoting policies that enhance integrated social development, social protection schemes. In the area of strengthening sustainable management of the natural environment, UNDP helps the Government of Egypt to build its capacities and develop adaptation options and local solutions to climate change, besides promoting biodiversity.

The UNDP programmatic priorities in Egypt's transitional period focuses on four major pillars: supporting expanded and effective political participation, supporting greater transparency and accountability, promoting a culture of human rights, and supporting local development, poverty reduction and social justice, which reflect the notion of governance.

Although all international and national actors work within the development policy framework of the state, yet they have their own agendas that serve their visions and missions. Additionally no coordination among all these actors, except some cases, for instance at national level the MLD implements infrastructure projects while the Ministry of Housing, Utilities and New Communities do the same. Some donors implement their projects in collaboration with governmental bodies in order to gain access to targeted areas and people, especially now as people are susceptible towards such organizations and their hidden agendas. As mentioned when preparing the SAD's document towards 2030 which reflect national objectives, it was done in coordination with Agricultural Research and Development Council, FAO, with inputs from IFAD and WB.

### **Conclusions**

In Light of the previous literature it is evident that “governance” is not applied regarding the overall state policies and plans in general and ARD in particular, besides there are no direct rural development strategy as it is included briefly within the agricultural strategy, even though enhancing the agriculture sector will eventually lead to improving those involved in the agriculture yet no improvement in quality of life of rural people, which is the core of RD. Additionally, socio-economic state plans are scattered among various ministries and authorities that creates duplication and lacks coordination, also this is the case in the international organizations dealing with ARD.

Therefore the paper recommends changing in both policies, legislations and institutions relevant to ARD, though ancient formation of the state plan that depends on centralization, without participation of relevant actors should be changed, and Ministry of Agriculture should be responsible about ARD policy in Egypt or Ministry of Local Development to be changed and take that part with involvement of various actors. Additionally, local people representatives should participate in any ARD policy to reflect their basic needs, yet their awareness about “governance”, is required so they can set their priorities on real basis. Research and extension should be empowered and practice their role with farmers, but this require changes in structure of the current institutions and budget to be suitable to carry on this role. Finally farmers should be gathered in one entity and fragmentation of agricultural lands should be based on collective production.

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**IMPROVING MAIZE SEED ACCESS AND CULTIVATION PRACTICES OF  
SUBSISTENCE FARMERS IN NUSA TENGGARA TIMUR, INDONESIA**

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**Abstract**

Access to quality seeds and technical know-how markedly increased maize productivity of subsistence farming communities, contributing to increased food security for at least 18,000 households in 2012. The paper aims to describe how to improve maize cultivation practices for subsistence and poor farmers by introducing quality seed and improving capacity of extension staff in Timor Tengah Utara (TTU) District of Nusa Tenggara Timur, Indonesia. A cooperative approach between national and sub-national Indonesian Government agencies, and the Australian Government's aid agency (AusAID) was the main key to achieve these. Firstly, the Indonesian Government's National Seed Reserve Program (CBN) distributed 198 tons of Open-Pollinated-Variety (OPV) seed to 427 farmer groups in 106 villages across TTU district. Secondly, recognizing the limitations of district agricultural services to disseminate knowledge and understanding of better farming practices, the AusAID supported the District Government and NGOs to undertake an intervention in 2011/2012 to improve the effectiveness of the CBN Program, thereby increasing food security for small-holders farm communities. The results of the intervention showed that the use of the OPV seed improved maize productivity by 36% (from 2.6 t/ha to 3.5 t/ha) or increased by between 52-86% when cultivation was managed through Demonstrations Plots compared to local varieties used by farmers. Capacity building of field facilitators (Public Extension and NGO's staff) to help disseminate knowledge and an understanding of the improved technologies was provided through Training of Trainers and a number of other knowledge-sharing exercises.

**Keywords:** Maize, food security, capacity building, sustainability, Indonesia

**Introduction**

Maize is a staple food of the majority of the population, and among the major crops cultivated by farmers in the Nusa Tenggara Timur (NTT) province of Indonesia. Despite its importance and the potential of the province for rural and agricultural development (Barlow, 2006), maize cultivation is characterized by low input-low output subsistence farming practices that are consistently unable to meet food demand due to low productivity caused largely by limited use of agricultural inputs such as improved seeds and fertilizer, and technically equipped extension services to assist improved farmer practices (Swastika *et al.* 2004). A community needs assessment activity in the region revealed that many poor farmers are trapped in a spiral of dependency due to subsistence farming systems practices (World Food Program, 2009). The low incomes earned from agricultural activities is barely enough to buy food for the 3-4 month gap in grain supplies normally experienced before the next maize harvest in most NTT households (YMTM, 2007). Farmers in NTT tend to cultivate maize only on a piece of land, normally 0.2 to 0.5 ha, that they can manage with their own

family labour, using traditional practices. This size of cultivation, however, is not sufficient to feed the household throughout the year, partly due to low-yielding-variety crop management practices, as well as substantial post-harvest losses due to weevil attacks. Research trials have shown that by using better seeds and farming practices, crop productivity will increase significantly (Bora and Murdolelono, 2006; Panikkai, 2009, Fargher and Kelly, 2012). However, most farmers in NTT recycled their own seeds from the previous harvest, a practice that eventually results in declining yields.

The Indonesian Ministry of Agriculture (MoA), under its mandate to increase maize productivity, has provided a subsidy for high-yielding-variety seeds to farmers across the nation through the National Seed Reserve (CBN) Program under the Directorate General of Food Crops (DGFC). The maize seed distributed through the CBN Program in 2011 was 4,600 ton nationwide (Ministry of Agriculture, 2012) and the Timor Tengah Utara (TTU) district of NTT received 198 tons of OPV maize seed. Factors that hindered the effectiveness of the program are lack of technical assistance, low technical capacity of extension services in the district level and lack of agricultural input supply, as well as the delayed arrival of seed in the area. Recognizing these limitations, the Australian Agency for International Development (AusAID) support the District Government of TTU and local Non-Governmental Organizations undertook an intervention to improve the effectiveness of the maize distribution program.

## **Material and Methods**

### **The Approaches of Technology Demonstration and Dissemination Intervention**

The intervention design was discussed involving the DGFC, the local government of TTU, local NGOs, farmers' representative and AusAID. The technology intervention components introduced for improving maize production system were: introduction of high yielding open-pollinated-variety (OPV) of maize seed, planting density arrangement, increased cultivation area (from 0.5 to 1 ha), introduction of agricultural inputs (organic and inorganic fertilizers, pest management products), and post-harvest storage management. One hundred and ninety eight tons of OPV maize (Surya variety) was distributed to 427 farmer groups in 106 villages in TTU starting in early December 2011.

Farmer Field Schools (FFS) through the establishment of demo-plot aimed to disseminate technical know-how of maize cultivation and to increase farmers' skills and capacity. Seventy demonstration plots were established across the district and technical assistance was provided by expert from agricultural research and service delivery institutions to improve farmers' and field facilitators' skills. The FFS apply the necessary crop management practice, as agreed prior to the activity, and was managed by the NGOs field facilitators, public extension staff (PPL) and farmer group members. Cross visits of farmers (member and non-member groups) to the demo-plot sites were held to disseminate the maize technology being introduced. Field visits were conducted at different demo-plot sites and growth stages of the crop growing period, i.e., emergence, flowering and maturity.

The size of demo-plot is one hectare which was divided into 4 small plots (0.25 ha each) and treated with four different cropping system to allow farmers to learn the know-how of maize cultivation. The allocated cropping systems were as follows: (A) OPV maize seed, monoculture, planting distance 40 x 75 cm, application of organic fertilizer; (B) OPV maize seed, monoculture, planting distance 40 x 75 cm, application of inorganic fertilizer; (C) OPV maize seed, integrated with pulse (peanut), planting distance of (100-50)x40 cm, application of in-organic fertilizer; and (D) OPV maize seed, integrated with pulse, planting distance

(100-50)x40 cm. The respective application of an-organic fertilizer was 200 kg urea + 200 kg NPK/ha, and organic fertilizer was 2,000 kg/ha.

The economics of maize production was analyzed to assess the profitability of using new OPV seed and modern management practices over existing practices. The important variables considered were seed variety (local vs. OPV), fertilizer used, labour allocation and other management practices.

Capacity building was carried out for 275 field facilitators (Extension staff or PPL and NGO's field facilitators) through Training of Trainers (TOT) and undertaking efforts to ensure knowledge-sharing. More than 200 farmers were interviewed for their perception of technology introduced to them in the crop season of 2011/2012. Pre- and post-training evaluations were conducted to assess the improvement of their knowledge.

Post-harvest storage management was done by introducing hermetic drum storage to reduce post-harvest loss due to weevils. The project provides 70 drums, one drum in each demo-plot to try out how to use the drum for grain and seed storage.

## **Results and Discussion**

### **Maize Productivity**

Access to modern high-yielding variety seed and technical know-how markedly increased maize production and productivity of subsistence farming communities, providing increased food security for at least 18,000 HHs (or approximately 90,000 people). The improved varieties gave higher grain yield compared to the local variety. The average grain yield obtained from the demonstration plots were 4.5, 4.8, 4.2 and 3.9 t/ha for cropping systems of A, B, C, D, respectively, as detailed in Table 1. In comparison, the grain yield from the local variety and using existing farmers' practices was 2.6 t/ha. Field observations was also carried out on farmers who received OPV seed and planted it in their own farm, with limited fertilizer inputs (some used organic fertilizer, some none), the average grain yield was 3.7 t/ha (n=55). Table 1 shows the grain yield observed during the activity.

The provision of airtight food-grade storage of polyethylene (hermetic) drums enables farmers to protect the grain from weevil and rodents and reduces the grain loss. The grain stored in the drum was not attacked by weevil at all until the following crop season of 2012/2013. The traditional method of grain storage by smoking and hanging the maize cobs in the 'Lopo' (traditional storage hut) does not provide adequate protection against weevil. Studies conducted by deRosari *et al.* (2001) show that 20 to 50% of grain is lost to pests over the course of 9 months of storage. A study conducted by the World Bank found that grain storage using an airtight drum has showed reducing grain harvest-loss and eventually reduced seasonal variation in food consumption, anticipated food shortages and improved lean season health (Basu and Wong, 2012).

### **Economic Analysis of Improved and Local Practices**

In the non-demo-plot practice, farmer participants employed similar planting distance practice to that of demonstration plots, but only a few farmers applied fertilizer. Among those who applied organic fertilizer the rate varies from approximately 500 kg to 2 t/ha, but none of them applied

Table 1. Grain yield at demo-plot sites and farmers' sites.

Observation	Yield (t/ha)		Number of samples (n)
	Maize	Peanut	
<u>Demo-plot sites:</u>			
(A). OPV, demo-plot, monoculture, organic fertilizer	4.5		47
(B). OPV, demo-plot, monoculture, in-organic fertilizer	4.8	-	47
(C). OPV, demo-plot, integrated maize + pulse, in-organic fertilizer	4.2	0.17	47
(D). OPV, demo-plot, integrated maize + pulse, organic fertilizer	3.9	0.14	47
<u>Farmers' sites:</u>			
OPV, non-demo-plot, monoculture, limited agricultural inputs at farmers' plot	3.7	-	55
Local practice using local variety seed (control)	2.6	-	694

in-organic fertilizer. In the local practice, farmers use local seed varieties, traditional planting distance practices (100 x100 cm or wider), apply no fertilizer, and sow 3-5 seeds per hole. By using quality seed, it was found that a farmer could gain an additional IDR 747,500/ha. Further, by applying fertilizer in a monoculture system, an additional income of IDR 2.7 – 3.4 million would be obtained (Table 2). There was not much additional income generated from integration of maize and pulse, compared to monoculture. The plantation arrangement between maize and pulse and the quality of peanut seed contribute the lower marginal income of this system. Using the improved quality seed and better crop management would generate a marginal rate of return of > 120%, except the crop system of integrated maize + pulse with organic fertilizer which is caused by low yield of maize and peanut. This means that quality seed and crop management are economically feasible to be improved.

#### Capacity Building of Field Staff and Farmers

Training of Trainers improved the knowledge of the field staff. The basic agronomy skills of participants were evaluated before and after training to measure whether there was improvement of the knowledge. Some results of the training activity were:

- The average score achieved before and after training were 42.5 and 54.7%, respectively. While this shows some improvement due to training, it also shows that further improvement is needed.
- Female participants achieved higher scores than males (64.8% VS 52.4%).
- Public extension officers and NGOs Field Staff have comparable knowledge on maize agronomy (59.4% and 57.0%, respectively).

#### The Lesson Learned

During reflection on the activity, farmers and public and private extension staff provided very positive feedback about the initial outcomes, and also outlined areas for improvement. Initial key learning from the first year of intervention were:

- The participatory approach of all stakeholders from the beginning of the intervention produces a solid team to implement the activity. The public extension officer and the field officer of NGOs were working closely in assisting farmers on cropping practice.

Table 2. Marginal rate of return between improved OPV maize cultivation and local seed.

	Average Yield (t/ha)	Gross income* (IDR)	Seed and fertilizer cost (IDR)	Labour cost (IDR)	Gross margin (IDR)	Marginal income (IDR)	Marginal cost (IDR)	Marginal rate of return (%)
OPV, demplot, monoculture, organic fertilizer	4.5	11,250,000	1,025,000	3,860,000	6,365,000	2,747,500	2,002,500	137
OPV, demplot, monoculture, in-organic fertilizer	4.8	12,000,000	995,000	3,900,000	7,105,000	3,487,500	2,022,500	173
OPV, demplot, integrated maize + pulse, in-organic fertilizer	4.2 (maize) + 0.17 (peanut)	12,576,000	1,345,000	4,300,000	6,931,000	3,313,500	2,762,500	120
OPV, demplot, integrated maize + pulse, organic fertilizer	3.9 (maize) + 0.14 (peanut)	11,430,000	1,725,000	4,200,000	5,505,000	1,887,500	3,042,500	62
OPV, non-demplot, monoculture, limited agricultural inputs, at farmers' plot	3.7	9,250,000	475,000	2,900,000	4,365,000	747,500	492,500	152
Local practice using local variety seed (control)	2.6	6,500,000	62,500	2,820,000	3,617,500	-	-	-

\*) Gross income is the value of sales of the grain, valued at IDR. 2500/kg and IDR 12,000/kg for maize and peanut, respectively, at harvesting time. 1 US\$ = IDR 9,500.

- The combination of access to improved seed varieties, expert technical assistance and mentoring of extension staff improved crop yield and enhanced food security of the communities.
- Improved maize cultivation methods and using quality agricultural inputs will increase the marginal rate of return, and may feasibly increase incomes of subsistence farmers.

While the impact of the seed distribution was significant, the potential barriers to the adoption of outputs include:

- Farmers rely on the distribution of free maize seed by Government. The unreliable nature of government assistance means that seeds are often not to arrive in time for the planting season, decreasing the effectiveness of the distribution program in achieving higher maize crop productivity.
- No availability of OPV seed and erratic availability of other inputs in the local market. The OPV maize is not available from the local input suppliers in the district. Some retailers offered OPV seed stock a few years ago, but because the Government distributes OPV maize seed for free the commercial market for OPV seeds has disappeared. Furthermore, there was erratic availability of fertilizer in the market which meant the program was unable to apply inorganic fertilizer at full rates.
- Nothing for free. Lessons from past experience show that farmers who receive inputs for free attach little value to them, and they may not use properly or even not use at all. Free inputs also encourage dependency and distort market value of inputs.

#### The Way Forwards

To ensure sustainable access to quality seed and necessary technical assistance, it is necessary to build an intervention model that assists to develop markets for good quality seed, rather than farmers relying on annual Government subsidies, and encourages farmers to adopt better cultivation methods suited to their local conditions. Taking into consideration participants' reflections and interviews with beneficiaries and potential partners, the next stage of the project will build on the success of the seed distribution program, but will add a new set of

partners from the local private sector. The involvement of local private seed producers who are capable of supplying seed at affordable prices, available when needed and easy to access at the village level is required to accelerate the development process.

### **Concluding Remarks**

The distribution of good quality seeds coupled with capacity building support has significantly increased maize production in target communities. Program data shows that the use of the OPV seed improved maize productivity by 36% compared to local varieties (from 2.6 t/ha to 3.5 t/ha) at the farm level. When maize cultivation was intensively managed through Farmer Field Schools, productivity increased by 52-86% compared to local varieties.

Instead of the usual but unsustainable practice of handing out agricultural inputs for free, development should focus on building farmer's capacity to identify needs, review and test options, and make better informed decisions of what change would work for them. Simultaneously, services and inputs required by farmers to implement the change need to be made available, either through public or private service providers.

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**CONDITIONS AND PERSPECTIVES OF FINANCIAL LENDING IN  
MACEDONIAN AGRICULTURE AND RURAL DEVELOPMENT**

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**Abstract**

Agriculture is a fundamental economic activity in Macedonia. It constituted 10% of GDP over the past decade. Adding related processing industry increases this share to approximately 15%-16%. During the last period of economic restructuring, agriculture played a critical role in the social and economic stability of the country. As a generator of growth of income and employment (19% of the total employment) agriculture needs a steady stream of funds to increase the competitiveness of the farmers by improving the technological and market infrastructure as well improving quality of life of rural population. Besides the state financial support of agriculture and rural development, the commercial financing - through lending is from utmost importance as well. The pressure for acceleration process for developing competitive and sustainable agriculture and sustainable rural communities requires better access to the funds by farmers. In this regards, the purpose of the research is to explore the existing situation in the lending system in agriculture and further provide analysis and recommendations for improving the commercial form of financing to farmers. Therefore, the desk work and field research through questionnaire and interviews have been conducted in order to collect information about needs and problems of the key actors in the field of lending in the agriculture and rural development. The results have been communicated with the stakeholders at panel discussion and, furthermore, upgraded and distributed to the relevant institutions and organisations. Findings demonstrate that the lending system in agriculture does not operate in an ideal environment and is facing numerous problems and obstacles.

**Key words:** *lending, agriculture and rural development, financial institutions, farmers.*

**Introduction**

The main challenges of the agriculture in the Republic of Macedonia in the path of EU accession are to harmonize its legislation with the EU *acquis communautaire*, adapt its institutions and economic policies, strengthen the rule of law and develop market oriented economies. During this integration process, the agro-food sector as a whole faces problems with development of competitiveness, creating market institutions, establishing marketing and distribution chains, meeting EU quality, veterinary and phytosanitary standards.

Credit as a financial instrument is one of the basic tools for vital agricultural development and has a crucial role in the development of profitable production. Lending allows additional accumulation of capital required for investments in the consolidation of agricultural holdings, purchasing of new equipment and machinery, modernization of production and increasing productivity (Kovacev et al., 2013).

Provision of financial services for agriculture and rural development is rather limited. The rural financial system consists of commercial banks, micro-credit banks, savings houses, informal financial channels and considerable credit-oriented programs financed by donors. Lending in agriculture is considered as high risky since its performance depends on many factors such as: climate and nature, marketing, legal, institutional, human factor, technical, financial, macroeconomic and political factor. According to the data from National Bank of Macedonia (NBRM), borrowers in the agricultural sector share is 19.9% in the highest risk groups of credits D and E compared to a share of 8.7% in other sectors. The assessment of credit requirements for investments in agro-industrial complexes is based on certain assumptions about the technical and production parameters, forthcoming demand and prices of products, cost and availability of raw materials and management skills of the borrower. The risk assessment is particularly important in terms of setting an upper limit on borrowing and defines the other terms of the loan. Uncertainty about forthcoming trends of these parameters over a longer period of time and the impact on the viability and profitability of the project on certain external variables complicates the credit analysis.

#### Specialized Credit lines from the Macedonian Bank for the Development Promotion to support agricultural sector

The Macedonian Bank for Development Promotion (MBDP) is a development and export bank whose strategic goal is to provide support and to incite development of the Macedonian economy through providing finance to small and medium-sized enterprises and export-oriented companies. The Bank which operates in line with the strategic policies, goals and the priorities of the Republic of Macedonia offers two the most favorable credit lines in the agro-food industry.

I) **Agricultural Credit Discount Fund (ACDF)** is a specialized credit line aimed at providing continuous participation in the financial sector in lending to agro-industrial complex by creating a model for refinancing of the loans. The credit line ensures supply of affordable loans to individual farmers and agro-food industry for activities in *primary production, processing industry* and *agro-exports*, with opportunity to cover costs for fixed assets and working capital for the above mentioned 3 credit categories. Lending to the target group is made through selected financial institutions that are 10 commercial banks and 2 saving houses. Interest rates of the credits offered by commercial banks amounted to 4% per year for primary production and 5% per year for investments in processing industry and *agro-exports*, while interest rates through saving houses are 6% and 6.5% per year, respectively. Credit terms such as repayment and grace period, fees etc. are identified by financial institutions according to their own credit policy depending on the type, purpose and amount of the loan.

II) **Credit line for production, processing and export of agricultural products.** The goal of this credit line is improving and developing production capabilities of micro, small and medium enterprises whose core business is the production, processing or export of agricultural products. Target group are only micro, small and medium enterprises, unlike the previous credit line for which individual farmers are also eligible applicants. With this credit line both fixed assets and working capital are financed. This credit line offers the best interest rate of the Macedonian capital market with only 3% per year.

#### Materials and methods

This research was conducted in the period November 2012 - February 2013 and consists of three phases: i) a survey on the farm level, ii) meetings and discussions with financial

institutions and iii) a panel discussion with stakeholders in the agricultural sector for communication of the primary results.

In order to facilitate the data collection for the first phase, a questionnaire was designed and a field survey was conducted in three regions in Macedonia: Kocani, Bitola and Gostivar through organized workshops in each region, structured to be attended by farmers with different farm sizes and agricultural subsectors. Workshops were attended by 100 farmers and qualitative and quantitative data has been collected. Additionally, many farmers were interviewed individually with supplementary set of questions mainly covering the issues of investments and access to finance.

In the second phase meetings with 5 financial institutions were held and discussions about their views and experiences in relation to credit arrangements directed to agriculture, the risks they face, and some recommendations for farmers who have to follow when applying for credit were conducted.

The third and final phase aimed to sit down stakeholders together and the results of this survey were presented in order to bring their postures towards improvement of lending to agriculture which will be of mutual interests for farmers and financial institutions.

Apart from field collection of data, the research is also based on secondary sources of data mainly from Ministry of Agriculture, Forestry and Water Economy, Statistical office, National Bank of the Republic of Macedonia, Macedonian Bank for Development Promotion, Ministry of Finance.

### **Results and discussion**

The field research, interviews and discussion with stakeholders gave ground to describe the typical features of the situation with lending to agricultural sector in the Republic of Macedonia. General perception of the stakeholders is that the banking sector is much more oriented towards urban areas where there is a greater concentration of potential customers and industries with higher profit margin (services, trade and heavy industry) than the rural areas that are predominantly oriented to agricultural production.

Credit support from financial institutions as a source of funding covers short-term loans with repayment periods of up to one year offering the financing of working capital (seed, fertilizer, fuel, pesticides, animal feed etc.) and long-term loans with deadlines for returning of more than one year and intended to finance investment projects (production facilities, agricultural land, irrigation systems, establishment of orchards and vineyards, supply of livestock, purchase of equipment and machinery for finalization of agricultural production, introducing new production lines to export). Till end of 2012, through ACDF, 5,501 projects were financed with total amount of €67 million, of which €37.2 million were approved in primary agricultural production, €25.7 million in the processing of agricultural products and €4.1 million to support the export of agricultural products. The biggest share of credit investments in primary agricultural sector is in livestock production which constitutes 21%, furthermore is the share of investments in establishment of orchards and vineyards makes 14%.

Graph 1: Approved loans breakdown by agro-food subsectors.  
Sources: Ministry of Finance, 2013.

Lending in this sector is mostly focused on larger farms so that a number of smaller individual farmers have **limited access to credit**. The majority of farmers (70%) consider that they are not sufficiently respected by financial institutions or are treated as incapable to use credits. Some farmers stated that financial institutions require more documents unlike other applicants who are not dealing in agricultural sector, or sometimes are being manipulated by the banks about the level of interest rates or administrative costs. In terms of awareness, more than half of the interviewed farmers answered, they do not have information on specialized credit lines in agriculture.

Foremost farmers' problem to meet criteria is to provide **collateral**, since the banks rarely accept property in the rural areas such as houses and agricultural land. Other problems farmers face to provide property deeds needed for collateral due to unsolved property rights. Unrealistically assessment of property as subject to the granting of collateral is a supplementary problem that farmers experienced. Additionally farmers mentioned that securing the guarantors becomes problematic because nowadays is getting harder to find them.

Other constraint of lending in agriculture is the **seasonal character** of the agricultural activities and incomes, which basically implies the requirement for adaptation of credit terms depends on seasonal needs for funds and ability to meet obligation to repay the loan corresponding to income flow. Farmers generally have a problem with the payment of annuities, stating that its dynamics should be adapted to the seasonal nature depending on the type of production, that is when the greatest income from agricultural activities. An additional problem that complicates the regularly payment of annuities is the delay of the payment to farmers by buyers. According to the graph 2 which illustrates monthly frequency of the number of requests for credits within ACDF credit line, providing a larger volume of liquid funds for lending by financial institutions in the season (March and December) and relaxation of liquidity out of season (July and August) is required.

Graph 2: Number of requests for credit from ACDF credit line for the period 2004-2012.  
Source: Ministry of Finance, 2013.

During the panel discussion, approach of creating a credit policy of banks based on a flexible rate versus fixed interest rate that is applied to all banks was considered. It was assumed that costs for loans with fixed and flexible interest rate are roughly the same but the dynamic amount of payments is in line with the capabilities of the farmers.

Table 1. Comparison of loan disbursement with fixed and flexible interest rates.

Simulation of loan disbursement with fixed interest rate					Simulation of loan disbursement with flexible interest rate				
Year	Principal	Interest	Annuity	i (%)	Year	Principal	Interest	Annuity	i (%)
1	0	400	400	4	1	0	200	200	2
2	0	400	400	4	2	0	200	200	2
3	1,250	400	1,650	4	3	1,250	200	1,450	4
4	1,250	350	1,600	4	4	1,250	350	1,600	4
5	1,250	300	1,550	4	5	1,250	300	1,550	6
6	1,250	250	1,500	4	6	1,250	375	1,625	6
7	1,250	200	1,450	4	7	1,250	300	1,550	8
8	1,250	150	1,400	4	8	1,250	300	1,550	8
9	1,250	100	1,350	4	9	1,250	200	1,450	10
10	1,250	50	1,300		10	1,250	125	1,375	
	10,000	2,600	12,600			10,000	2,550	12,550	

Source: Own calculation

According to the responses in the questionnaires, given that agriculture is low profitable sector; favorable **interest rate** should range from 2% to 7%, with most mentioned affordable interest rate (43% of the interviewed farmers) to be equal to 4% (graph 3).

Graph 3: Recommended interest rate by farmers.

Source: Field survey, 2012.

Research shows that another limiting factor for lending to agriculture is the absence of **credit histories** of borrowers since they do not practice running of records of all agricultural activities and costs and incomes on the farm. This situation leads to lack of adequate information which hinders the development of quality business plan or credit analysis by financial institutions.

## Conclusion

Access to credit resources for rural and agricultural activities is very limited since the financial institutions are quite reluctant to invest in them as agriculture is viewed as a risky or highly risky sector, where a majority of farmers do not perform bookkeeping, there is lack of secure title deeds to be used as collateral, insurance premiums are expensive and therefore not used regularly, rural enterprises are generally micro and small-scale, with low managerial and administrative skills.

It is of great importance for farmers in order to improve production and market competitiveness to be well informed for availability, terms and conditions of available specialized credit lines in agriculture. Given the nature of the activity performed, and the areas in which they exist which are mostly rural and distant from urban centers, the best way to be better informed and to have influence in creating of national credit policy in agriculture is approaching associations, federations and cooperatives. Financial institutions also may need to intensify the mobility of credit administrators through the implementation of mobile offices on different days and in different rural areas to promote credit lines for farmers and assist in the preparation and collection of credit documentation.

One of the most restrictive factors in terms of lending is unregulated property rights and unregistered properties in the official cadastre, what disable providing collateral for using of loans. Because of that, farmers need to resolve property issues and record their own property in the national cadastre that will lead to increased possibility of getting loans and acquisition of rights to use the program for financial support of agriculture and rural development. We must also make clear that farmers who conduct accounting evidence are in better position to use credit from financial institutions.

Financial institutions need to design credit products for farmers with longer period of repayments and credit packages with variable interest rate (no more than 4% per year on average) and variable repayment terms or flexible repayment schedule. For example, interest on loans for establishment of orchards and vineyards may be lower in a grace period of three years till achieving full productivity, and later in full exploitation of the perennial crops can be offset by a reasonable higher interest rate.

However, the fact is that farmers and financial institutions have the same goal - the maximization of profits, which is not possible without mutual cooperation, understanding and confidence. Additionally, agriculture and rural development should be in focus of national policy makers to assist improving the lending system.

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## NITROGEN FERTILIZERS IN THE MEDITERRANEAN REGION: USE TRENDS AND ENVIRONMENTAL IMPLICATIONS

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### Abstract

Humans have caused unprecedented changes to the global nitrogen cycle. The increased use of nitrogen as fertilizer has allowed increasing food production for a growing world population but has had also considerable adverse effects on the environment and human health. The paper aims at analysing nitrogen use trends in the Mediterranean region. The work is mainly based on secondary data from different databases such as the FAOSTAT and the World Development Indicators (WDI). Two main indicators were considered: fertilizer consumption and nitrogen fertilizers use. According to the WDI, nitrogenous, phosphate and potash fertilizers consumption increased in the period 2002-2009 in almost all Southern and Eastern Mediterranean countries. Fertilizer consumption in the Mediterranean ranged in 2009 from 7.8 to 502.8 kg per ha of arable land, in Algeria and Egypt, respectively. However, there was a general decrease of nitrogen fertilizers consumption between 2002 and 2010 in Mediterranean countries with few exceptions (*e.g.* Egypt). Nevertheless, nitrogen use is still high *e.g.* 388.2 kg N/ha of agricultural land in Egypt (2010). High nitrogen use is exacerbated by food waste; which implies the loss of large amounts of resources and inputs, such as fertilizers. About 80% of nitrogen used in food production is lost before consumption and the remainder is lost as human waste. Nitrogen lost to the environment affects water quality, air quality, greenhouse balance, ecosystems and biodiversity, and soil quality. These negative externalities call in question the dominating agricultural development paradigm and food production system sustainability.

**Keywords:** nitrogen fertilizers, Mediterranean region, environment

### Introduction

Environmental degradation has reached in the Mediterranean proportions that require immediate action (UNEP, 2010). Much of today's discourse about environmental problems revolves around reducing greenhouse gas emissions and water usage. According to Rockström *et al.* (2009) stratospheric ozone, biodiversity, chemicals dispersion, ocean acidification, land system change, atmospheric aerosol emissions and nitrogen and phosphorus discharge should also be considered.

The present food system delivers low cost food at a high cost to the environment (Kickbusch, 2010). Food costs include also environmental impacts of food production, distribution and consumption (Ingram, 2011). Therefore, the need to improve the efficiency of inputs, such as nitrogen, in agricultural systems is well recognised (*e.g.* Ahrens *et al.*, 2010). Nitrogen is an essential nutrient for the growth of plants. Although nitrogen occurs naturally and is essential for life, human activities are releasing excessive and polluting amounts into the environment (Leach *et al.*, 2012). The largest sources of nitrogen pollution come from food production and the burning of fossil fuels (Erisman *et al.*, 2008). Reactive nitrogen derives mainly from artificial nitrogen fertilizer production through the Haber-Bosch process (Woods *et al.*, 2010).

Of all the chemical elements, nitrogen is one whose abundance has been increased the most by human activity (Erisman *et al.*, 2008). Over the past century humans have caused unprecedented changes to the global nitrogen cycle (Sutton *et al.*, 2011). The total amount of reactive N created by human activities has increased ninefold over the last 100 years due to the increased use of fertilizers (Millennium Ecosystem Assessment, 2005). In the last 50 years, global use of artificial nitrogen fertilizer has increased seven times (FAO, 2011). Present global food supply depends on artificial nitrogen fertilizer. Globally, around half of the nitrogen in soils comes from artificial nitrogen (Liu *et al.*, 2010).

The human use of reactive nitrogen (Nr) has profound beneficial and detrimental impacts (Leach *et al.*, 2012). The beneficial impacts of the agricultural use of Nr are related to food production using nitrogen fertilizer and human-enhanced biological nitrogen fixation (Erisman *et al.*, 2008). Artificial nitrogen fertilizers have allowed increasing crop yield (Ibarrola-Rivas and Nonhebel, 2011). The detrimental impacts occur because most of the Nr used in food production is lost to the environment where it causes a cascade of environmental changes that negatively impact both people and ecosystems (Sutton *et al.*, 2011, Leach *et al.*, 2012). The paper aims at analysing fertilizers and mineral nitrogen use trends in the Mediterranean region. It also investigates the environmental implications of excessive nitrogen use.

### **Material and methods**

The paper is mainly based on secondary data from different databases such as the FAOSTAT and the World Development Indicators (WDI) of the World Bank. Two main indicators were considered: fertilizer consumption and nitrogen fertilizers use. The geographical coverage of this study is similar to that of the Mediterranean Strategy for Sustainable Development (UNEP/MAP, 2005) including 11 Northern Mediterranean Countries (Albania, Bosnia and Herzegovina, Cyprus, Spain, France, Greece, Croatia, Italy, Montenegro, Malta and Slovenia) and 10 Southern and Eastern Mediterranean Countries (Algeria, Egypt, Israel, Lebanon, Libya, Morocco, Palestinian territories, Syria, Tunisia and Turkey).

Fertilizer consumption measures the quantity of plant nutrients used per unit of arable land. Fertilizer products cover nitrogenous, potash and phosphate fertilizers (including ground rock phosphate) (World Bank, 2013). Data are available, on a yearly basis, for the period 2002-2009 and are sourced from the World Development Indicators (World Bank) based on elaboration of FAOSTAT data.

Mineral nitrogen consumption accounts for nitrogen input that implies the use of nitrogen fertilizers in agricultural production. It is calculated as the average quantity of mineral nitrogen (in kg) used per hectare of national agricultural land. Data are available - on a yearly basis - from FAOSTAT-Resources database for the period 2002-2010.

### **Results and discussion**

During the period 2002-2009, fertilizers consumption ranged from 6.0 kg/ha of arable land recorded in Algeria (2003) to 696.6 kg/ha recorded in Egypt (2008). In the same period, average fertilizer consumption ranged between 11.6 kg/ha recorded in Algeria and 563.0 kg/ha recorded in Egypt. Fertilizer consumption in Egypt is even 4.5 higher than that recorded in the European Union. That can be explained by the fact that almost the whole arable land in Egypt is irrigated and agriculture is intensive. In the period 2002-2009, the average fertilizers consumption in the 21 target Mediterranean countries was 188.0 kg/ha so higher than the worldwide average (116.3 kg/ha of arable land). During the same period the average fertilizers consumption in the Middle East & North Africa (90.6 kg/ha) was lower than the levels of fertilizers consumption in the Euro area (179.9 kg/ha) and the European Union countries (155.5 kg/ha).



Considering the same period, fertilizers consumption decreased in almost all target Mediterranean countries. It slightly increased only in Egypt, Cyprus, Montenegro, Tunisia and Turkey. The highest decrease was recorded in Lebanon and Slovenia (Figure 1).

Figure 1. Trend of fertilizer consumption in the target Mediterranean countries, 2002-2009. Data are not available for the Occupied Palestinian Territories (OPT).

In the period 2002-2010, mineral nitrogen consumption ranged between 0.1 kg/ha recorded in Algeria in 2005 and 468.9 kg of mineral nitrogen/ha of agricultural land recorded in Egypt in 2003 (Table 1). In the Mediterranean region 52.9 kg N/ha are used on average. During the same period, the highest average nitrogen use was recorded in Egypt (373.0 kg N/ha) while the lowest use was recorded in Algeria (0.9 kg N/ha).

Table 1. Mineral nitrogen consumption in the target Mediterranean countries (kg of mineral nitrogen/ha of agricultural land). Data are not available for Cyprus and the OPT.

Country	2002	2003	2004	2005	2006	2007	2008	2009	2010
Albania	33.3	33.1	31.9	34.2	26.8	27.7	23.8	27.9	25.7
Algeria	0.7	0.4	2.5	0.1	1.1	0.9	0.6	0.5	1.1
Bosnia	8.8	5.0	15.5	11.4	7.2	10.0	5.5	10.7	8.4
Croatia	109.9	103.3	123.7	103.1	155.4	176.7	204.4	135.0	50.1
Egypt	312.5	468.9	396.4	416.7	293.9	312.7	441.2	326.8	388.2
France	74.5	80.0	78.5	74.6	74.7	81.7	71.6	65.2	70.3
Greece	32.2	62.2	27.0	51.3	20.9	29.1	21.3	16.1	17.9
Israel	79.4	91.1	102.0	94.8	93.4	114.6	89.0	69.1	58.4
Italy	55.3	56.8	58.2	54.3	56.2	57.4	46.4	34.9	34.8
Lebanon	31.9	26.8	27.4	13.4	11.8	19.5	14.7	19.0	23.6
Libya	4.9	2.1	3.0	4.8	2.4	4.4	2.7	3.0	3.0
Malta	59.8	50.1	64.3	62.2	100.7	61.4	36.5	41.9	32.3
Montenegro	-	-	-	-	3.4	2.7	2.6	2.1	2.7
Morocco	8.2	6.7	7.7	11.2	10.7	10.2	9.2	6.5	4.5
Portugal	42.6	28.9	33.0	26.9	23.2	30.3	25.7	26.8	34.4
Slovenia	65.3	66.7	60.9	56.7	61.0	58.7	50.6	59.9	56.1
Spain	34.9	41.1	36.9	31.7	33.9	35.2	26.3	27.9	34.2
Syrian	16.1	17.5	16.3	19.3	19.9	18.9	19.4	15.1	8.6
Tunisia	3.8	5.4	4.9	6.3	6.6	4.0	5.9	7.8	6.9
Turkey	29.1	33.0	33.2	33.3	34.7	34.4	29.0	36.3	34.4
<i>Mediterranean</i>	<i>54.5</i>	<i>61.8</i>	<i>57.2</i>	<i>56.4</i>	<i>50.0</i>	<i>53.7</i>	<i>54.7</i>	<i>44.7</i>	<i>43.4</i>

In the period 2002-2010, average mineral nitrogen consumption decreased in the Mediterranean area from 54.5 to 43.4 kg of mineral nitrogen/ha of agricultural land. Mineral nitrogen consumption decreased in all Mediterranean countries except in Algeria (+0.4 kg N/ha), Tunisia (+3.1 kg N/ha), Turkey (+5.3 kg N/ha) and Egypt (+75.7 kg N/ha). The highest decrease was recorded in Croatia (-59.8 kg N/ha).

Mineral nitrogen trade balance (export - import) is negative in the Mediterranean area. Considering the period 2002-2010, mineral nitrogen trade deficit increased from -1,275,809 to -1,441,037 tonnes of nitrogen *i.e.* +165,228 N tonnes. As of 2010, almost all the Mediterranean countries are net mineral nitrogen importers except Israel, Jordan, Tunisia, Croatia, Libya, Morocco and Egypt. The top net mineral nitrogen exporters are Egypt (1,381,065 tonnes of nitrogen) and Morocco (327,023 tonnes of nitrogen). Meanwhile, the top net mineral nitrogen importers are France (-1635,446 N tonnes), Turkey (-967175 N tonnes), Italy (-409540 N tonnes), Spain (-369243 N tonnes) and Greece (-124780 N tonnes).

High fertilizers, especially nitrogen ones, use and imports as well as their increasing prices without forgetting price volatility - as fertilizers prices are strongly linked to the energy prices - puts at risk food availability and affordability and calls in question the environmental as well as the economic sustainability of the current Mediterranean food consumption patterns.

Excess in nitrogen represents a serious risk to the environment. The increased use of  $N_r$  as fertilizer has considerable adverse effects on the environment and human health (N-Print, 2010). Of the N used to produce food, about 80% is lost before consumption, and the remainder is lost after consumption as human waste (Sutton *et al.*, 2011). The major risk is that nutrients, particularly nitrate, will run off into surface water or percolate into groundwater. Moreover, some nitrogen, is lost to the air through volatilisation of ammonia (a contributor to acidification) or as nitrous oxide ( $N_2O$ ) (a powerful greenhouse gas) (Pau Vall and Vidal, 1999). Reactive nitrogen affects water quality, air quality, greenhouse balance, ecosystems and biodiversity, and soil quality. Nitrogen fertilization leads to the contamination of drinking water, algal blooms, eutrophication, etc. (N-Print, 2010). Once lost to the environment, nitrogen moves through the Earth's atmosphere, forests, grasslands and waters causing a cascade of environmental changes that negatively impact both people and ecosystems. These changes include smog, acid rain, forest dieback, coastal 'dead zones', biodiversity loss, stratospheric ozone depletion and an enhanced greenhouse effect (Galloway *et al.*, 2008). The deposition of N, P and other contaminants is expected to have an impact on biodiversity. Nitrogen pollution damages ecosystems and affects human health, including respiratory diseases and the risk of birth defects (N-Print, 2010) and causes local environmental problems such as eutrophication, biodiversity loss, soil acidification, and ground and surface water pollution (Eickhout *et al.*, 2006).

Excessive nitrogen surpluses can pose a threat to the environment. The integration of environmental concerns into agricultural policies and practices can help reducing pollution. Nitrogen losses to the environment can be minimised if a reasoned fertilization is used, together with sustainable agricultural practices, such as crop rotation, planting cover crops, and ploughing in crop residues. Reasoned fertilization means applying fertilizers in the correct weather conditions (to avoid run off) at the appropriate stage in crop growth (so that plants take up the nitrogen quickly) and at the correct doses (Pau Vall and Vidal, 1999).

Nitrogen-related environmental footprint is exacerbated by food losses and wastage. It is estimated that 10-15% of non-perishables (*e.g.* grains) and up to 60% of perishables are lost during the whole production chain. In addition, post-cooking losses are also significant (FAO/RNE, 2011). Wasting food means losing not only life-supporting nutrition but also precious resources, including land, water and energy. Food losses and waste imply that large amounts of land, energy, fertilizers and water have also been lost in the production of foodstuffs which simply end up as waste (Institution of Mechanical Engineers-UK, 2013; FAO, 2012). Reducing the amount of food wasted throughout the food chain in the entire Mediterranean area would contribute to easing pressure on natural resources. According to Ingram (2011), reducing waste across the whole food system will increase the amount of food available for human consumption for the given level of inputs, thereby improving input use efficiency.

### Conclusions

Over the past decade, great progress has been made in communicating to the public the role that their actions have on the carbon and water cycle. This did not happen yet in the case of nitrogen. Essentially all the reactive N created is lost to the environment, where some portion accumulates in soils, waters, biomass and the atmosphere. Nitrogen footprint increases as a result of artificial nitrogen fertilization, manure runoff and the burning of biomass. Anthropogenic reactive nitrogen increases ozone and particulate matter; increases the acidity of soils, streams and lakes; changes the ecosystem productivity; increases global warming

potential; and decreases stratospheric ozone. Nitrogen can contribute to each of these environmental changes in sequence thus affecting ecosystem integrity and human health.

The average fertilizers consumption in the Mediterranean countries is higher than the worldwide average. Fertilizers consumption in the European Mediterranean countries is still higher than in the Middle East & North Africa. Nevertheless, fertilizers consumption in Egypt is among the highest in the whole Mediterranean. In general, fertilizers consumption decreased in Mediterranean countries during the last decade.

In the period 2002-2010, average mineral nitrogen consumption decreased in the Mediterranean area. Nevertheless, it remains high in many Mediterranean countries especially those that rely on intensive irrigated agriculture (*e.g.* Egypt). The Mediterranean region is a net mineral nitrogen importer due mainly to the negative nitrogen trade balance of Northern Mediterranean countries and Turkey.

It is crucial to link the reactive nitrogen losses in agricultural fields to their effects on the environment. For this reason there is a need to estimate the losses to the environment in terms of emissions to the air (ammonia and NO<sub>x</sub>) and the run-off and leaching to groundwater and rivers (nitrate) in the different Mediterranean agro-ecological zones. Additionally, this should be linked to the cascading nitrogen effects to provide quantitative estimates of the relationship between the virtual N and the environmental impacts. Nevertheless, it should be highlighted that the assessment of the impact of nitrogen use should not be limited to the agricultural site, as large improvements in the nitrogen cycle can be achieved by changing food consumption patterns and reducing food losses and waste along the food chain.

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**INSTITUTIONS AND RURAL DEVELOPMENT IN TRANSITION ECONOMIES  
WITH SPECIAL EMPHASIS ON THE REPUBLIC OF MACEDONIA**

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**Abstract**

Analysis begins with view of the impact of institutions on economic development in rural community as given in the theoretical concept of the new institutional economics with clearly pointed distinction of the different institutional levels, although both are important for transition economies and the Republic of Macedonia.

The paper emphasizes the importance of embedded institutions for the transformation of the rural economies in the transition countries and identifies and analyses institutional aspects regarding rural development common to the transition countries. It focuses on the institutional dimension of reform policies, i.e. on the institutional economy aspects of the transition process that were decisive for the success or failure of the reforms.

Studying the case of Macedonia shows that reform and transformation of the rural sector are caused inter alia by the failure of previous rural development institutional concept. The new institutional concept developed for establishing new institutions in rural institutional environment and strengthening property rights and expectations for a variety of rural sectors in terms of enhancing their efficiency and competitive position.

**Key words:** institutions, rural development, rural community

**Introduction**

Williamson (2000) distinguishes four levels of social analysis of institutions. The most basic set of constraints that shapes human behaviour in the society forms the so-called ‘embedded institutions’, which Williamson suggests as the first level of social analysis. Examples include traditions, cultural beliefs and religions. The second level consists of the rules of the game and in particular, how property rights are defined and established. The third level constitutes the play of the game, i.e. how the rules from the second level translate into actions of the economic agents. The traditional focus of institutional economics lies in the second and third levels of social analysis. The neoclassical analysis belongs, in Williamson’s classification, to the fourth level of social analysis.

This article starts from the premise that an economy’s transition requires large-scale institutional change. Moreover, “there are vast domains of institutional transformation that cannot be achieved simply by the dictates of a proclamation from the central government” (Stiglitz, 2000). Institutions are highly important, even if the term is not always well defined. North (1990) suggests a clear distinction between institutions and organizations: institutions constitute the rules of the game, while organizations, which are groups of individuals bound by common objectives, are comparable to the players in a game. Such a distinction is helpful in framing institutional problems. In contrast to North’s definition, the World Bank (2002) suggests that the term “institutions” refers to both rules and organizations. Recognizing the close interaction between institutions and organizations, this article utilizes North’s definition

of institutions, as is commonly done in institutional economics, but includes the analysis of organizations as well. Both are important for transition.

Some institutions may have emerge partly from a specific culture in a given society and have evolved over time; others have been formally introduced by a society as a whole or by some of its members in order to facilitate exchange. Finally, some important institutions may have been introduced informally by certain special interest groups in order to improve their particular well-being. The informal institutions may be in conflict with formal institutions and may reduce their effectiveness. Embedded institutions also affect the functioning of formal institutions, yet they have been largely neglected by western economists when giving advice to transition economies. Research over the last decade has shown that the same set of formal rules may have different consequences depending on the economic and cultural situation (Huntington, 2000) in the country at the outset of transition. According to Harrison (2000), progressive cultures emphasize the future; static cultures emphasize the present or past.

This article will emphasize the importance of embedded institutions because they may be of special concern for transforming the agricultural sector and rural economies given that rural societies are often more constrained by inherited rural sector in socialist conditions as well as tradition and cultural values in transition economies as in Macedonia. Also research special attention paid to the institutional environment and various specific features that it incorporates the South Eastern European Countries (SEECs) countries and Macedonia.

Nevertheless, it is possible to identify some privatization policies in transition phase and role of institutions in SEEC countries. Assessing the weakness of specific institutions requires referring to specific countries. In transition economies, at an early stage, the main emphasis of institutional changes placed on privatization and strengthening property rights while in the R.of Macedonia attention is given to the second stage - the creation of new institutions and organizations. In both cases, due to lack of data and empirical studies, the general hypotheses posed for a large set of countries, can not be fully tested and proven exact. Taking into account this fact, findings should be taken as an attempt to further clarify the important role of institutions in rural development and by no means as definite findings about the examined aspects. In the case of the R. Macedonia we are forced to rely on author's previous multi-research in this field, which in part can be used to extract information about the topic set.

### **Privatization policies in transition phase and role of institutions in SEEC countries**

In the early transition phase it was widely expected that the SEECs would quickly restructure their agricultural sector towards family farms. Those expectations have not been fulfilled. It is important the role of institutions for the governance of farms, the comparative advantage of farm sizes and the choice of specific legal forms of farms.

People could no longer rely for decision-making on the hierarchy in place during socialist times; instead, they had to take responsibility upon themselves. Socialist legislation that impeded changes in large farms delayed the birth of privately-owned farms. Labour legislation and issues concerning corporate governance of large farms were extremely important. Those SEECs that dismantled the old farm structure either by restitution or by allocating property widely among the population suffered less from the legacy of the people's socialist behaviour. In particular, labour legislation and the old style of public and private governance obstructed the restructuring of the agricultural sector less when the old farm structure was dissolved at the very beginning of the transformation process.

The mode of privatization may have created a strong lobby for securing property rights if the new owners feel that they could use their property more efficiently if it were

better protected. This outcome can only be expected if privatization has led to private ownership with personal direct use of the property. If privatization has led to collective ownership or private ownership that is used collectively, there will be less lobbying for securing property rights. It is not a surprise that the SEECs, which created private ownership in the first years of transition, enjoy more highly secured property rights in agriculture than other countries.

In that context, past policy decisions have affected the structure, conduct and performance of political markets. Privatization policies created new stakeholders interested either in further policy reform – mainly by securing property rights – or in blocking further reforms by inhibiting further private ownership in agriculture:

- The mode of privatization has affected the political market for policy reform and has had an impact on the change in mental models;
- The creation of new collective farms (i.e. those farms which succeeded the former collective and state farms) has given birth to new players in the political market and has strengthened some players while weakening others. This concerns farm managers, regional governments and the central government;
- The lack of an adequate system of public finance, which would permit financing the social health and education systems in rural areas, has created stronger support for the survival of the large-scale farm sector in the SEEC;
- The strength of individual players in the political market is dependent on, among other things, the performance of the agricultural sector. Political support for the sector is easier to obtain if its income situation is rated badly;
- The new institutional environment created by the mode of privatization and the establishment of the new collective farms increased the income-earning capacity of the managers in most cases.

### **Institutional changes and institutional environment of rural sector in Macedonia**

Restructuring of agricultural enterprises complexes as large agri-industrial firms and companies that handled much of the agricultural land was required before privatization, or being sold separately, according to their individual activities. The logic of this kind of privatization and restructuring was the need to increase efficiency and competitiveness in the sale of business units, i.e. of certain small parts of agro industrial enterprise. In addition, 15% of agricultural land was in possession of a large agro industrial enterprises were taken and given to the free sale of private farmers. The aim of this measure was to encourage the development of the land market in Macedonia.

Land was partially revoked by the Law on Agrarian Reform Law of 1945 and the nationalization in 1953 and agricultural policy in Macedonia was aimed at changing property relations in agriculture, which was considered a non socialist sector and was not responsible for the development of productive forces. In an effort to abolish private sector and restore state capitalism did not allow farmers to dispose of own machinery. The main objective of the reform and privatization was mainly strengthening property rights to improve economic efficiency.

Building new institutions in rural sector – second level of institution

Legal and institutional framework

Currently there is no systemic legal act for horizontal regulation of the agricultural sector and rural development.

### Agricultural and rural sector legislation

- Law on Agriculture and Rural Development;
- Legislation on the budgetary means for agriculture development;
- Legislation on agriculture land;
- Legislation on support of rural areas;
- Legislation on setting up registers in agriculture.

### Institutional framework

1. The Ministry of Agriculture, Forestry and Water Economy (MAFWE),
2. The Agency for Financial Support in Agriculture and Rural Development
3. The Ministry of Local Self-Government, the Ministry of Transport and Communications, The Ministry of Environment and Physical Planning, and other state institutions.

### Strategic documents

1. The National Strategy for Agriculture and Rural Development for the period 2007-2013
2. The Action Plan for Agricultural Development and Action Plan for Rural development

Awareness of the rural policy makers about the importance and opportunities of the rural sector and expectations of such policy

Below are some of the important issues related to awareness of the rural policy makers and their expectations.

1. Awareness of the importance of the role of agriculture and food industry in the national economy;
2. Awareness to create competitive production and increase income of the rural population that is one of the preconditions for social stability in the country;
3. Awareness decline in agriculture, forestry and fishery and associated industries could have significant adverse consequences in rural areas and to the overall economic and social stability of the country;
4. Geographic diversity in culture, customs, traditional events;
5. Lower education levels of rural population;
6. Absence of relevant rural organisations to represent the interests of rural communities and promote training;
7. Strengthening of civil society institutions and increased awareness of the benefits of the cross-sectoral partnerships;
8. Weak interest of the general public and lack of empowerment of inhabitants in finding solutions to rural problems;
9. Strengthening property rights;
10. Build an efficient agricultural sector that will be competitive and to contributes to economic development;
11. Increase income and improve the living standards of farmers;
12. Increasing the competitiveness of agriculture in order to its successful EU integration;



13. Strengthening farmers' organizations;
14. Optimal use and management of natural resources in an environmentally sustainable way;
15. Institutional strengthening of public institutions and increasing quality of services in the agricultural sector;
16. Integrated rural development policy;
17. Possible risks of (non)- implementation or insufficient quality enforcement activities.

Achieved awareness at institutional level is reflected in the design of government's agricultural and rural policies to support agriculture and rural sector (Programme for Financial Support of Agriculture and National Program for Rural Development) - established a second level institutions. Lack lays not so much in their implementation, but the conceptual framework in which a significant portion of the development is transformed into social policy of rural areas.

Rural institutional environment - overcoming some aspects of political and cultural obstacle

In practice, the importance of political structure and public culture for rural development in Macedonia entails a number of difficulties. To begin with, rural communities can be very slow to recognize that they are in crisis. The obvious risks of change often blind rural community members to the real costs of maintaining the status quo or some of them think that it is better to return to the old (socialist) system.

Second, the mere existence, and even perception, of shared rural community interests in change may not suffice to precipitate in the creation of needed public structures and strategies. Rational self-interested individuals will not necessarily act collectively to further what are in fact their shared interests. The welfare of the community is a public good that, like many others, will tend to be suboptimally supplied.

To help close this gap, a structure of organization and leadership is needed that can authoritatively allocate selective benefits to individual members of the community. But this functional requirement is not a "demand" that automatically generates its own "supply"; public as well as private entrepreneurship may be in short supply.

Finally, the emphasis on the importance of public culture and beliefs can bring more disappointment. It is easy to think of these variables as external and resistant to rapid change - in particular, through deliberate transformative efforts. For decision makers is far easier to deal with problems of infrastructure and capital rather than become involved with policies designed to alter a population's behavior and perceptions.

Solutions. What are the alternative ?

1. A crisis can become so acute that the rural community can no longer deny its existence, cost/benefit perceptions may shift massively against the status quo, and cultural constraints on change are decisively weakened. But while this option is important analytically, its policy relevance is less evident; it is difficult to imagine politically acceptable ways of engineering crisis for the purpose of promoting needed change.
2. The internal forces needed to overcome cultural and institutional obstacles can be decisively strengthened through appropriately structured external incentives (as the meaning and effects of the IPARD program and national government rural program).
3. Even in the absence of external incentives, local mobilization sparked by the perceived importance of local rural communities and places can make a real difference.

### Conclusion

Institutions are largely country-specific, it is possible to identify a larger group of countries' institutions that are crucial for the transformation process. For example, all transition countries suffer from reforms that do not meet the expectations created at the outset of transition. Hence, the institutional aspects of the transition process would be crucial for the success or failure of the reforms. It is important to keep in mind, though, that institutions can be changed.

Owing to, in the SEECs rural people could no longer rely for decision-making on the hierarchy in place during socialist times; instead, they had to take responsibility upon themselves. The mode of privatization may have created a strong lobby for securing property rights if the new owners feel that they could use their property more efficiently if it were better protected. Further, are defined the important issues related to the institutional environment such as: past policy decisions that have affected the structure, conduct and performance of political markets, policy reform. The lack of an adequate system of public finance and the strength of individual players in the political market.

In the R. Macedonia, unlike SEECs, the main objective of the institutional reform and privatization was mainly strengthening property rights of previous small landlords to improve economic efficiency and privatization of large agri-industrial firms. Furthermore, need to continue building new institutions in rural sector ( as second level of institutions) by the creation of new legal and institutional framework and better defined expectations of the new reformed agrarian system.

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## **SUSTAINABLE DEVELOPMENT AND THE ENVIRONMENT PROTECTION PROBLEMS**

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### **Abstract**

The environment has been considered as a free good in previous development, so it has been mercilessly exploited, which had left and keep leaving multiple negative consequences. Economic development, based on irresponsible use of natural wealth cannot last forever, and not to jeopardize a capacity of our planet's perseverance. Old development models must quickly change, if we want to provide a long-term integrity of natural systems, which keep life on Earth. In order that economic development move into the future according to the ecological requirements, more and more talks about the necessity of, so called, sustainable development, raising awareness on importance of healthy environment, which enriches its content with ecological, social and other dimensions. This developmental concept is opposite to the previous/existing philosophy and strategy of a fast growth, based on a “wasteful” and “dirty” technology with brutal environment exploitation. The sustainable economic growth has to and must be an engine of the ecological progress. In order to achieve the sustainable economic growth, i.e. the growth adjusted to the nature needs and limitations, we have to provide the connection between the ecological and economic policy on all social levels and in all economy sectors.

**Key words:** sustainable development, ecology, competitiveness, socially responsible business.

### **Introduction**

In 1987, the World conference for the environment protection and development presented to public its report “Our Common Future”, known as “Brundtland report”, after the name of the president, who chaired the Commission. This report had given a great stimulus to discussion on criteria and terms of sustainable development, as a new concept of the world economic and ecological policy. The sustainable development as a possible stage toward a main goal of the global survival requires a thoroughly rearrangement of ecological performance of all economic activities. A specification and defining the concrete tasks can be found in the *Business Charter for Sustainable Development* (1991), the Second World Industry Conference on Eco-Management. The most of set requirements have already entered the existing eco-management systems (BS 7750, EVRO-EMAS, ISO 14000). Privatization and restructuring of companies make the basic terms for sustainable development. In the Report of the European Bank for Reconstruction and Development for 2005, the transition in Serbia was highly rated (Porter, 2004). However, the production of companies in Serbia insufficiently fulfils the market requirements regarding quality, prices, assortment, delivery terms and the organic production standards. Low capacity utilization and impossibility of a product placement are a consequence of business philosophy, by which the production is a goal by itself, where an ecological dimension of production is pushed into the background. Such attitude toward economy had brought to increased stocks, which had additionally increased already high production costs. A significant factor of a new sector development and the sustainable development in Serbia is an affirmation of socially responsible business. It is

about a relatively new concept, which has been very efficient in terms of the global competition. The socially responsible business concept contributes to realization of economic success, along with a respect of individuals, society and environment interests. There enables the resources allocation which appreciates the “*triple bottom line*” criteria (economic development, social development, responsible relation to the environment). Maintaining a delicate balance between industrial development and the environment preservation leads to redefining of the existing resources combinations, due to new costs of transition to the environment-friendly technologies (Djuricin, 2006). An assumption of the socially responsible business concept is free adoption of measures which contribute to resolution of social and ecological problems. It is about a strategic approach to the society and a partnership with the other companies in the environment.

### **Material and working method**

In realization of the research task was used a desk research of data regarding the sustainable development and the environment protection issues. Such research implies using the data from the official resources: reports of the United Nations, the EU regulations, domestic and foreign literature; internal documentation database of the Institute of Agricultural Economics, Belgrade. Accordingly, there can get more reliable answer to key questions, which arise within the analysis of the sustainable development and modern challenges in the field of the environment protection. New investment methods of decision making require establishing relation between criteria location of economics, socials and ecologicals factors. Management that respects situation approach in practice can gain balance of economic efficiency, social responsible work and environmental standards.

### **Results and discussion**

With occurrence of new challenges of globalization, significant interest has been directed to development of new methods for solving diverse business influence in the society. Many of those influences are far-reaching and profound. Just to mention some, as:

- **Environment pollution** caused by production, transport and using products as cars, refrigerators or newsprints.
- **Increasing problem of waste disposal** and its management as a result of excessive packaging.
- Devastating results for individuals and local communities, due to **closure of the local companies**, reduction of their size and number of employees, which is an experience well known in Europe, from South Wales in Great Britain, to the countries of Central and East Europe.
- **Erosion of local cultures and surroundings** owing to a penetration of mass tourism in places like: Mallorca fishing villages, Swiss alpine communities, as well as ancient Roman monuments.

After the *Earth Summit*, held in 1992 in Rio de Janeiro, a concept has imposed as the one which should be at full length applied (although it had not been unilaterally accepted). It is about a new conceptual frame for evaluation of not just business activity especially, but also industrial and social development generally. That concept is the *sustainability concept*. The sustainability has started to be a common expression in a rhetoric regarding a business ethics, so its uses in full length by corporations, governments, consultants, scientists, etc. Despite the wide use, sustainability is a concept which is used and interpreted in different ways (Dobson,

1996). Probably the most common use of the expression is related with the sustainable development, which usually defines sustainable development as: *Development that meets the needs of the present without compromising the ability of future generations to meet their own needs* (World Commission on Environment and Development, 1987).

This definition, however, represents only a basic idea of a widely deniable expression, which was also a subject of enormous series of different conceptualizations and definitions. Thus, along with all the caution against an unquestioning acceptance of any interpretation, on a basic level, it seems that sustainability primarily refers to the system preservation, as well as to the fact that our activity does not affect the systems (for example, Earth or biosphere) in a way that their long-term sustainability is jeopardized. Focusing on the sustainable development and the future generations potential to satisfy their needs, sustainability also faces reflections on inter-generational capital, i.e. on equality between one generation and another. For a long time the sustainability concept has substantially stayed a synonym for human environment sustainability. Recently was yet enlarged not only to the environment considerations, but also to the economic and social development (Elkington, 1998). This sustainability concept enlargement has emerged not only for the reason that it is impractical, sometimes even impossible, to analyze the sustainability of natural surroundings without the consideration of social and economic aspects of relevant social communities and their activities. The second argument for this consideration continuation is if the capital should enlarge to the future generations, then it is logical that it also should be enlarged to those in the existing generation. Thus, one of the primary goals set by the World Commission for Environment and Development is to uproot the world poverty and inequality. As it is noticeable, sustainability can be observed through three components: ecological, economic and social. It refers to a definition: “*Sustainability refers to long-term system preservation in accordance to a man’s environment, economic and social development.*“ (Djuricin, 2006). Maintain the subtle balance between industrial development and environmental conservation leads to redefine the existing resource combinations due to the costs of transition to new technologies friendly to the environment. Although the definition is meant for determination of a basic content of sustainability concept, it is obvious that sustainability as a phenomenon represents a specific goal, which should be achieved. Shaping the sustainable development as the goal of the business activity of a company is the most complete expressed through a “triple bottom line” concept. The “triple bottom line” is a term which has been formulated and strongly pleaded for, by John Elkington, director of the Consulting Agency for Sustainability Strategies and the author of many esteemed books on corporate environment. His opinion on this concept was based on the fact that it represents an idea that business is not related only to one goal which reflects in maximizing economic value, but he added the enlarged set of goals, which implies including the environment and a wider social community. From this point of view it should be clear why sustainability is a new goal, potentially important for business ethics. However, in order to develop clearer picture on what these three sustainability components represent for the business ethics, one must analyze each of them.

***Perspectives related to the environment protection.*** The sustainability concept is generally considered that it has emerged from the environment protection perspective, especially in forestry, and later also in other areas of resources management. Indeed, in this moment there is still quite a widespread perception in business (although we believe it is wrong) that sustainability is often a concept of man’s environment protection. Thoroughly sustainability principle in the perspective of the man’s environment protection comprises an effective management of physical resources, in a way to be preserved for the future. All bio-systems

are observed as the ones with limited resources and finite capacity, and thereby also the sustainable human activity must work at the level which does not jeopardize endangered species health. Even at the most basic level, these problems point out to a need to eliminate a certain number of critical business problems, like influences of industrialization on biological diversity, further use of non-renewable resources as oil, steel and coal, as well as the production of harmful environment polluters. Although those relations also increase the economic growth issue, as a vexed question remains if the future generations could really realize the same life standard as us, without cancellation of increasing production and consumption trend. Consequently, ecological factor gets its significance, which makes a demand for special consulting services related to projection and installation of equipment for reduction/prevention of pollution (Cvijanovic, Mihailovic, 2010).

***Economic perspective.*** The economic perspective of sustainability has emerged from the economic growth model, which assess capacity limits of the planet Earth. Understanding that a continuous growth of population number, industrial activity, resources utilization and the environment pollution can lead to the life standard reduction, have initiated the sustainability occurrence as a way of thinking which would insure that the future generations will not be in an unfavourable position due to activities and choices of the existing generation. In time has expanded the opinion on improvement of sustainability macroeconomic comprehension. Such opinion implication on the business ethics have appeared at different levels. Narrow concept of economic sustainability focuses only on economic performances of the corporation. The management is responsible only for development, production and market of those products which ensure long-term economic performances of the corporation, without respect for the environment and society requirements. Paying bribes or forming cartels, for example, can be considered economically unsustainable, while these activities jeopardize long-term functioning of the market. The corporations, which try to avoid paying taxes via subtle accounting tricks, can say that they behave unsustainably: if they are not ready for financing the institutions which preserve the social surrounding and the man's environment (as schools, hospitals, police and justice system), then they jeopardize one of the key institutional grounds of their corporative success. Accordingly, there are forms of associations of researchers and activists of mutual interest (for example "International Pressure Group", "Tax Justice Network", etc.) on issues which serve for raising the awareness and stimulating actions against harmful influences of tax payment avoidance, tax competition and, so-called, tax havens.

***Social perspectives.*** Development of the social perspective related to sustainability usually comes after the ecological and economic perspectives and stays relatively new, as a phenomenon. Explicit integration of social problems into the business discourse about sustainability could be seen as a phenomenon during the 90s, and, first of all, seems as answer to a worry regarding the business activities influence on autochthonous communities in less developed countries and regions. Introduction of social reflection within the sustainability area has characterized a significant shift in this concept's interpretation. A key question in the social perspective is the one concerning the social justice. Regardless to an impressive progress of life standard, the UN, during 2005, had issued the *Report on Social Situation in the World*, which had identified a constant deepening of inequality on the planet. With 80% of the world gross domestic product, which belongs to one milliard people living in developed countries, and the rest 20% shares 5 milliard people who live in developing countries, as it is stated in the Report, was suggested that "*solving the inequality must secure a social justice and better life conditions for all the people, which is unachievable in this moment, so the communities, countries and regions are still vulnerable to social, political*

*and economic subversion*” (The Report of the World Social Situation, 2005). More concrete, in the Report is stated that there is “*an increasing chasm among qualified and unqualified workers, the chasm among formal and informal economy, as well as significant differences in health care, education and opportunities for social and political participation*” (The Report of the World Social Situation, 2005). Business, as one of the main starters of economic development, has been more and more present in such discussions. That is to say, fairer world, whether among rich urban consumers in the West and poor workers in developing countries, among the rich urban and rural poor, or among women and men, remains the essential issue of the sustainability social perspective. There remains an open question in which way should business respond to such challenge, but at least the goals got some explanations in last several years with declaration “The United Nations Millennium Development Goals”. These items, which will quote, represent the main social and developmental challenge which upsets the world at the moment and articulate the specific goals and indicators of what should be achieved until 2015. The eight “Millennium Development Goals” are: to eradicate extreme poverty and hunger; to achieve universal primary education; to promote gender equality; to reduce child mortality rates; to improve maternal health; to combat HIV/AIDS, malaria and other diseases; to ensure the environment sustainability; and to develop a global partnership for development.

Although the UN had determined the goals, they, in effect, represent a government responsibility which should achieve them. Some of them have very direct implications for business, while the others refer to wider environment in which companies have to do business. In regard to this enlarged set of expectations, which are put in front of business in accordance with the concept of “Triple Bottom Line”, there are many significant implications regarding the way in which should be observed the business ethics. Aiming to achieve sustainability in previously defined areas, maybe expects too much nowadays. There are negligible products, firms or industries which could reliably claim that they are sustainable in the full sense of the word. Nevertheless, with the concept of sustainable development widely promoted by governments, companies, non-governmental organizations and academic communities, which is obviously important in order to understand full implications and evaluate the business ethics application, at least according to potential contribution to sustainability. The programs of support to the sustainable development (primarily rural development) can have more significant effects if they are directed to competitiveness, innovations and employment in rural areas. By introduction of the rural development local strategies (LEADER approach), through stimulating interest of the local population by their active participation and creating the local action groups (LAG), the social capital in these areas can significantly increase. The EU Agrarian Council adopted in 2005 a fundamental reform of rural development policy for the period 2007-2013 (Regulation EC No. 1698/2005). The new rural development policy has set exactly the LEADER initiative as a leading approach in distributing support and has notably simplified the procedures, and these are the following “axes” in the period until 2013: Improvement measures, i.e. increase of the agrarian sector competitiveness; improvement of the environment and rural environment through the support to land management; economic diversification and improvement of life quality in rural areas; and the so-called, “horizontal” component or fourth axis of financing and conducting the rural development policy – the LEADER approach. Integrative approaches in the local development have been useful in the previous period, especially in sense of the local capacities construction and assistance to the government agencies and to the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. Positive influence reflects, first of all, in creating the adequate support measures and more qualitative assessment of the specific measures effects on the local development.

### **Conclusion**

Economic growth creates a capital for financing better ecological effects. The experiences of developed countries provide convincing evidence that the modern industrial world prosperity is important for ecological progress. Accordingly, the sustainable economic growth can and must be an engine of ecological progress. In other words, it can and must finance the technologies necessary for protection and purification of the environment. Today creates new, ecologically useful technologies, which changes a man and his environment interaction. Factories and equipment obsolete and replace with more productive, which simultaneously pollute the environment less. Namely, growth creates an expectation and the need to improve the environment. As the conditions of economic growth, income and living standards rise, people meet their basic needs for food, clothing and a roof, they can not afford paying attention to the quality of their lives and habitats. When you insure present can focus on their future

### **Acknowledgement**

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## **COST EFFECTIVENESS OF RASPBERRIES GROWING IN RURAL AREAS OF NORTH KOSOVO**

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### **Abstract**

Leposavic municipality is situated in the farthest north of Kosovo and Metohija, with 72 villages, about 5036 households and 14500 people. Agriculture has always been the main activity in this area. Livestock production is the main agricultural sector, but there are also good conditions for fruit growing especially for berries (raspberries, blackberries, blueberries). In Leposavic municipality raspberries are grown mainly in small rural estates from 10 to 30 acres in hilly and mountainous areas, with the best yield results. In those small estates there is no control over production which does not cover the minimum requirements of the customers. It is necessary to foster specialization of those households and to form associations in order to have full cycle raspberries production with mini-coolers that offer frozen raspberries as a final product. It would be important factor of sustainable rural development in the area of Leposavic municipality.

In order to achieve this goal, intensive raspberry growing is necessary. This paper presents an economic analysis guide to investments required to increase an area under raspberries plantations. An average calculation is given for raspberries growing in Leposavic municipality, as well as the profit and level of economic profitability. It is reasonable to expect that from the planned production, invested assets would be returned after three years which could form the basis for further development of the households, as well as the food processing industry.

**Key words:** raspberry, production, costs, economic importance

### **Introduction**

North Kosovo is the unofficial name for the region on the north of the Autonomous Province of Kosovo and Metohia mainly inhabited by Serbians. After Albanians declared independence North Kosovo remained under Serbian control. It consists of four municipalities: North Kosovska Mitrovica, Zvecan, Zubin Potok and Leposavic. Leposavic municipality is situated farthest north in Kosovo and Metohija, with 72 villages, about 5036 households and 14500 people. Agricultural production in North Kosovo is far behind considering real opportunities determined with agro-ecological conditions.

Obviously, institutional absence in terms of agricultural support creates an environment, for farmers, that does not provide real opportunities in agricultural production. There is no strategy for sustainable exploitation of natural resources in terms of their valorization and direction towards creation of an excellent source of employment for increasing unemployed population. The extension service that has the most important role in such process does not functioning or does not exist. So the goal of this paper is to draw attention on good conditions for fruit growing especially for berries growing (raspberries, blackberries, blueberries), before all in order to actuate a discussion about the need for a systematic approach to model formulation of agricultural development in the North of Kosovo. Years of experience from many regions in Serbia show that raspberries' growing (in good agro-ecological conditions)

is more profitable than other agricultural industries (Veljkovic et al., 2003). In Leposavic municipality raspberries are grown mainly in small rural estates from 10 to 30 acres in hilly and mountainous areas with the best results.

Investment in raspberries growing comes from the fact that there are good climate and other conditions for this kind of production in rural areas in North Kosovo. Previous experiences show that raspberry growing is profitable and that demand exceeds supply. This production is an ideal investment and good income of mixed households in hilly and mountainous areas of North Kosovo for the following reasons:

- ideal conditions for raspberries growing
- simple and easily managed technology of production and care
- economical and highly cost-effective production
- relatively small investments

### **Material and methods**

Plots for growing raspberries are at an altitude of 400 – 500 m on hilly and mountainous areas of Leposavic municipality. Weather conditions are very favorable with more precipitation, which is very good for raspberries, because of moisture in soil and in air. The most common cultivar in North Kosovo is Vilamet.

Present climatic and soil conditions are good for this cultivar with specific way of growing. Main characteristics of this cultivar are high yield, good quality and resistance to diseases and pests. With Vilamet cultivar spacing should be 2.5 x 0.25 m per hectare with 16000 raspberry seedlings. With Miker cultivar spacing should be 3.0 x 0.33 m with 10600 raspberry seedlings per hectare (Petrovic et al., 2002; 2005).

In order to achieve good quality growing of raspberries, seedlings should be good quality too, from registered nurseries, seedlings with well-developed root system with lots of small fibers with no symptoms of damage and illness. Planting should be in fall, with best reception, which ensures better growth during the next vegetation. North-South row direction is the best. In that way seedlings are in sunlit during the day (Petrovic et al., 2003).

Irrigation is necessary for modern, intensive raspberry growing (Veljkovic et al., 2006.), so it is necessary to incorporate in our investment calculation drop by drop irrigation system. Raspberry growing in family farms in North Kosovo was monitored during the past few years and based on data collected during that period investment calculation and calculation of raspberry growing has been made.

### **Results and discussion**

Raspberry production in the North of Kosovo is an important additional source of income for households, where the most of family members participate in the production process, even the youngest ones. So, investment decision about the land area with raspberry needs to be made according to the number of active household members, involved in the production process. Table 1 presents a calculation for raspberry growing on a family farm, 0.20 ha, using Vilamet cultivar with spacing 2.5 x 0.25 m.

Labor costs in the calculation constitute almost 50% of total production costs, which in turn give higher income for the household and increases importance of this kind of agricultural production particularly by increasing the employment rate. Given investment calculation contains predicted costs for growing of 0.20 ha of raspberries and total investment will be compared with annual income of raspberry growing in order to show economic viability.

Table 1. Investment calculation for raspberry growing

## A. Costs of material

No	Type of material	Unit of measure	Quantity	Price per unit	Price/Eur
1	Seedling	piece	3200	0.25	800
2	Manure	ton	10	20	200
3	mineral fertilizer a) NPK 8:12:26+3%Mgo b) KAN (29%N)	kg	140	0.35	49
		kg	60	0.30	18
4	Poles	piece	180	1.50	270
5	supporting poles	piece	50	1.00	50
6	wire and nails	kg	80	1.00	80
7	Drip irrigation system (approximate price)	piece	1	300	300
8	Pesticides	kg	1	90	90
9	TOTAL				1857

## B. Costs of service

No	Type of service	Unit of measure	Quantity	Price per unit	Price/Eur
1	Soil and agrochemical analysis	sample	2	35	70
2	Land flattening and clearing	hour/tractor	1	15	15
3	Manure transport	hour/tractor	2	15	30
4	Plowing	hour/tractor	1	25	25
5	Cultivation	hour/tractor	1	25	25
6	Furrowing	hour/cultivator	2	6	12
7	seedlings and fertilizers transportation	hour/tractor	1	15	15
8	Poles transportation	hour/tractor	1	15	15
9	spraying	hour/tractor	1	20	20
10	Processing between rows	hour/cultivator	4	6	24
	TOTAL				181

## C. Labour costs

No	Type of service	Unit of measure	Quantity	Price per unit	Price/Eur
1	ground preparations	working day	1	10	10
2	manure loading and unloading	“	1	15	15
3	manure spreading	“	1	15	15
4	raw marking	“	1	10	10

5	preparation of seedlings for planting	“	1	10	10
6	shortening and planting seedlings	“	2	10	20
7	watering	“	2	10	20
8	mineral fertilizer spreading	“	1	10	10
9	weeding and ground breaking	“	6	10	60
10	pruning and removal of cut shoots	“	1	10	10
11	back setting	“	8	10	80
	TOTAL				260

TOTAL (A+B+V) = (1.857 + 181 + 260) = 2.298 €

All the costs for one year of raspberry growing are presented in planned calculation (Table 2), where an average yield and purchase price is predicted, which served for the calculation of the profit expected.

Table 2. Planned calculation of raspberry growing (0.20 ha, planned yield 3,000 kg).

#### I Costs of material

No	Type of material	Unit of measure	Quantity	Price per unit	Price/Eur
1	manure	ton	3	20	60
2	mineral fertilizer				
	a) NPK	kg	120	0.35	42
	8:12:26+3%Mgo b)				
	KAN (29%N)	kg	60	0.30	18
3	pesticides	kg	1	80	80
4	binding	kg	2	5	10
5	TOTAL				210

#### II Costs of service

No	Type of service	Unit of measure	Quantity	Price per unit	Price/Eur
1	manure transportation	hour/tractor	1	15	15
2	mineral fertilizer transportation	hour/tractor	1	15	15
3	Cultivation (3x)	hour/cultivator	6	6	36
4	Spraying (5-6x)	hour/tractor	6	20	120
5	Transportation of raspberries	hour/tractor	8	15	120
	TOTAL				306

III Labour costs

No	Type of service	Unit of measure	Quantity	Price per unit	Price/Eur
1	manure spreading	working day	1	15	15
2	mineral fertilizer spreading	working day	1	10	10
3	tying and tensioning wires	working day	4	10	40
4	removal of young shoots (3x)	working day	4	10	40
5	hand-hoeing in row direction (2x)	working day	4	10	40
6	Harvest	working day	60	10	600
7	pruning and removal of old shoots	working day	2	10	20
	TOTAL				765

IV Amortization	185
V Other costs	60
A Total costs (I, II, III, IV, V)	1,526
B Production value (3,000 kg x 1.00 €)	3,000
V Profit (B-A)	1,474

Financial production indicators: profit (p) = production value (pv) - total costs (tc) (€)

Fruit cultivar	Raspberry
Production value	3,000
Total costs	1,526
Profit	1,474

$$\text{Production economy (E)} = \frac{\text{production value (V)}}{\text{total costs}} \times 100 = \frac{3000}{1526} \times 100 = 196.59\%$$

$$\text{Profitability rate} = \frac{\text{profit (p)}}{\text{investment (i)}} \times 100 = \frac{1474}{2298} \times 100 = 64.14\%$$

In the calculation there are also labour costs which are half of total production costs (765 €), and they remain in households as their profit. Household income is higher and economic interest is greater given that almost all family members participate and decrease the labor costs going outside.

### Conclusion

Based on economic analysis results of raspberry growing, it can be concluded that production generates income. Average calculation costs are predicted and production value is planned. Cost-effectiveness in raspberry growing is 196.59%. Depending of investments in raspberry growing, return rate is 64.14%. These calculations show advantages of raspberry growing in North Kosovo comparing to other fruit. Full yield will be in the 3<sup>rd</sup> year. With proper use of agro-technical measures, raspberry growing can be cost-effective from 12 until 15 years:

growing period lasts for 2 years, full yield period 8 years, decreasing yield period 5 years. Realized production and purchase price in the market directly affect profitability level.

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## FINANCING OF AGRICULTURAL EXTENSION SERVICES IN THE WORLD

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### Abstract

In modern conditions the role of agricultural extension services (AES), in addition to the transfer of innovation in agricultural production and farmers' education, is more diversified in accordance with the requirements of farmers, local development strategies and defined agricultural policy. Under conditions of limited funding by the state authorities, providing different services requested that AES becomes more complex and makes fertile ground for the transfer of part of the advisory activities to other actors, such as private companies and non-governmental organizations, which results in the change of traditional models of extension service organization. New organizational models and new approach to financing of extension services in agriculture are generated.

In general, there are two main sources of financing of extension services: *the state or the public*, which is implemented mostly from the agricultural budget or through a variety of projects funded by the relevant state institutions, and *private*, which exists particularly in the cases when part of the extension work is provided through private extension organizations, where payment for these services is directly or indirectly transferred to the final users – farmers. However, there are very complex models of financing of extension services in the world, which are most often a combination of these models.

In the paper will be given the analyses of the relationship between models of organizing and financing extension services, then the elements that influence the choice of funding model will be displayed, as well as connections between the objectives and the participation of certain actors in the financing of agricultural extension services. Flow of funds from source to end-users will be shown in public funding model, while in the private funding special attention will be given to the degree of coverage of user of extension services and various topics covered by the work of such organized extension services.

**Key words:** agricultural extension service, funding, sources, users.

### Introduction

The Agricultural Extension Service (AES) has an eminent role in the promotion of agricultural production, especially in countries in transition, where individual farmers are not only exposed to the changing agricultural policy, but also to increased foreign competition. At the same time, in developed countries, AES is highly organized and completely focused on meeting the specific needs of the user. In the world, therefore, there are differently organized and financed agricultural extension services. The applied model of organisation of these services depends on the agrarian structure, the level of technological development of agricultural production, socio-economic characteristics of its users, and available funds. In the early stages of development, AES is usually fully organized and funded by the government, while, during the economic empowerment of farmers, the burden for financing AES is transmitted to users, or clients. The transformation of the organization and financing of AES is accompanied by numerous changes in the scope of farmers, the quality of services provided and the subject areas covered by this service.

This paper analyzes the characteristics of different models of financing of agricultural extension services, and the effects of their work. The paper consists of three parts. The first section presents the methods used in the work and data sources. In the second section of the paper are analyzed two main sources of funding AES: state (public) and private, as well as various intermediate forms in which these two sources overlap. In the third part, conclusions and recommendations for the improvement of agricultural extension service in Serbia are presented based on the exposed experiences of other countries.

### **Materials and Methods**

The aim of this paper is to point out the various forms of financing agricultural extension services that exist around the world. Foreign literature is rich in theoretical studies of this topic, as well as images of certain forms of funding AES that exist in practice, while the domestic literature significantly deal with theoretical aspects of the organization and financing of this service. The primary method used in this paper is the analysis and systematization of the literature, while the comparison method is used in order to compare AES in Serbia and other countries. In addition to theoretical research methods, the paper shows empirical methods, especially when addressing the specificities of the organization and financing of AES in the Republic of Serbia.

The publications of international organizations that deal with a decades-long analysis of various aspects of agriculture are the main sources of gathered data, including agricultural extension service, such as the Food and Agriculture Organization of the United Nations followed by the Centre for Agricultural Extension and Rural Development in Switzerland. An important source of information was also publications and research papers from the national and foreign authors.

### **Results and Discussion**

Financing of the agricultural extension services cannot be considered separately from the forms of its organization, which can be conditionally divided into two main categories: state-organized (public) and private agricultural services.

Publicly organized extension service is usually related to financing from the state budget and its activities are predominantly covered by public funding. Generally, at the beginning of each fiscal year, one part of the total budget is directed for funding the agricultural extension activities. From the perspective of farmers, the services provided by such organized agricultural extension services are completely free of charge – because these are financed by the state. However, even in the state-organized extension service the cost can be transferred, more or less, to other funding sources, which are in the end directed to its final users. In other words, the state uses different mechanisms to relieve financial burden of extension services and to transfer the cost of its financing to other actors (Jankovic, 2007: 62). In addition to agriculture budget, which is the main source of funding in most countries with public AES, Rivera and Cary (1997) suggest alternative sources, such as:

1. Special forms of taxation of certain products which is targeted to most of advisory activities, such as, for example, coffee in El Salvador;
2. Differentiation of farmers and involvement of economically successful producers; and
3. Funding based on agreements and contracts, where farmers enter into contracts with public AES, as is the case in New Zealand.

One of the most commonly used model, especially in the early stages of commercialization of AES, is differentiation of farmers based on economic strength and the ability to bear one part



of the cost of extension services. In that way, richer farmers are included in financing AES, which means that they pay for provided services.

Basically, common to all these forms of extension services is that it is public, organized under the auspices of public authorities, for the most part financed by the state, while a smaller part of the cost is transferred directly (through contracts or paid advisory services to specific category of users) or indirectly (by introducing special forms of tax on certain agricultural products) to agricultural producers. In transition countries, where extension services are offered predominantly through a publicly organized AES (as is the case in Serbia), it is estimated that about five percent of extension work is entrusted to the private sector (Katz, 2002: 22).

At the present stage of development of agriculture, regardless of the socio-economic and social factors, there is no service that is exclusively organized through public AES. Ševarlic *et al.* (1999) reported that in Serbia in the period after the adoption of the Law on Professional Agricultural Services (“RS Official Gazette”, No. 61/91), apart from consultants of the agricultural services and agricultural experts employed in cooperatives and agricultural companies, part of the advisory services were provided by the veterinary services professionals whose services have almost entirely been charged from farmers.

In the future stages of the commercialization of agricultural extension services, in addition to the fact that the financial burden is being passed on to farmers, other providers of extension service are introduced. Basically, we are talking about the private consulting companies, companies producing and selling agricultural production inputs (seed, fertilizers, etc.), but also the organizations of producers, such as cooperatives, agricultural chambers and lately non-governmental organizations. In such circumstances, when part of the advisory services are transferred to private extension organizations, they may endure part of the costs, which is particularly important in terms of reducing agricultural budget, or at least the part directed to financing of AES (Nikolic and Arsenijevic, 2012: 832). However, even in almost completely commercialized systems of agricultural extension services, some services are implemented through public service. Ševarlic *et al.* (1999: 152-153) point out that in situations where the provision of extension services is transferred to private companies, public AES does not lose its significance.

As an antipode to the previous model, the main source of financing of such organized AES is the revenues paid by users – farmers, but an alternative source of financing can also be found, and they mostly depend on the type of advisory services and the areas to which they apply. The various mechanisms by which the state can help in providing extension services through private consulting companies are:

1. Vouchers and coupons systems, when the state approves vouchers for farmers to engage advisors as is the case in Chile or farmers getting a loan coupons, which are used for paying advisors engaged like in Colombia (Nikolic and Arsenijevic, 2012: 828); and
2. Payment of extension services through organizations of producers, where farmers do not directly pay a fee for consulting services, but they pay membership fee in agriculture chambers and then they pay for the services of advisors, partially from the charged membership fees, and partially from the funds received from the state budget. Participation from the state budget can be significant – in France, for example, the government contributes to 49 percent of these costs.

In the relevant literature it is a widely accepted opinion that there is no best model for the organization and financing of agricultural extension services (Jankovic, 2007: 70; Ševarlic *et al.* 1999: 153), but that the final form that will exist in a country depends on its specific

characteristics and takes into account characteristics of both agricultural production and the achieved level of development of rural areas, and farmers, as well as prevailing users of AES. Different models of organization and financing of AES are not mutually exclusive, and it is possible that in a country at the same time co-exist two or more different forms of services, such as in Germany, for example. The choice of the best adapted form of the agriculture extension services depends on many factors. Katz (2002: 14) states that the elements that define the financing of AES are:

1. Clients or users of extension services;
2. The extension services provided;
3. The context and framework conditions; and
4. The extension organizations that provide service.

*Clients of extension services* are primarily individual farmers, and other entities engaged in agricultural production, such as large-scale commercial farms, farmers' organizations (associations, unions, and agriculture chambers). Identifying of users of extension services depends primarily on who will benefit if the given advice is used in practice. In fact, if the goal of extension service is improving the quality of agricultural products, the main beneficiaries are farmers themselves, but intermediate users are purchasers and processors of these products. If the goal of the extension services is to reduce the use of chemical inputs, then the beneficiaries of these services are consumers, while in the case of hiring extension service to control for example ragweed, the user can be identified as the local population.

Depending who is the final beneficiary, it can be concluded who will bear the cost of hiring agricultural extension providers. As the number of potential users of the service increases, the source of funding is closer to the public/state funds. When the primary users of extension services are agricultural producers, then they will provide the greatest part or entire funding for extension services.

*The extension services provided*, or the relevance of discussed topical area is directly related to clients of extension service. Improvement of agricultural production, creating business plans, or protection from disease in livestock breeding is aimed at users – farmers, and extension service aims to increase their income, so they will participate or completely finance such a service. If provided extension service aims at improving the conditions of agricultural production in the local community, in addition to private resources of the farmers that may be the immediate beneficiaries of such a service, part of the funds can be raised from the local community.

In the case when it comes to consulting work in the areas where private extension organizations do not have a financial motivation, or cannot make a profit on the implementation of these activities, funding sources are shifting from private to public. As the topic of extension work is more general and number of users higher (such as the environment protection, rural poverty reduction, sustainable agriculture, ecological effects of agricultural production, etc), the motivations of users for participating in the financing is significantly reduced, because the use of these services is not limited only to clients, but to the community. In this case, the financing of such extension activities will be largely out of the public budget, regardless of who performs these extension activities.

*The context and framework* of extension services depends on the agricultural structure, the status of agriculture in the national economy, legal and institutional environment, etc.

*The providers of extension services* are agricultural extension organisations. There are different categorisations of extension organizations (Nikolic and Arsenijevic, 2012; Jankovic, 2007; Ševarlic *et al.*, 1999), and according to Katz (2002: 17) they can be divided into four categories: (1) state (public) organizations (such as AES in Serbia or state universities engaged in providing extension services as in the USA), (2) private profit-oriented organizations (consulting firms) (3) private non-for-profit organizations (NGOs), and (4)

producers organisations (cooperatives, unions, associations, community organizations, etc.). Regardless of the form of organization, all of these services can be financed by public and private funding sources, as previously discussed.

There is a direct correlation between funding sources and clients/users, or the part of the population that will benefit from the implemented extension activities. Rivera and Cary (1997: 249) stated that, models of AES can be grouped into three categories: (1) public funding for part of the extension services that benefits the entire population, (2) payment of extension services for activities that will provide a direct increase of farmers income, and (3) mixed funding for services where the end-users are farmers and residents of local communities.

Regardless of the form through which extension service is implemented, flow of funds from the state authorities to farmers could be twofold: direct – from the source of funding, or the state institutions to extension organizations that then provide services to clients, and indirect – from source of funding to farmers, who then directed it to the extension organizations of their choice. The second model is commonly found in circumstances where great number of organizations is dealing with the extension services or when in addition to public there are private extension organizations. In the literature this model is often referred to as institutional pluralism and provides greater competition between providers of extension services, which often results in higher quality of services that farmers can get. Therefore, the decision to appoint the extension providers is up to the final user, as well as the flow of funds from the state budget to extension organizations.

There are very complex models of financing the AES in the world. In general, we can say that commercialized extension service suits the needs of farmers better, and that they can get higher quality of extension services. When farmers participate in the financing of extension services, their representatives have more influence on its development, leading to a further improvement of the quality of the AES. At the same time, the shift to commercialized extension means that a smaller number of farmers will use extension services, primarily those who are economically stable and able to pay. This results in a further polarization among farmers. It also reduces the dissemination of knowledge among farmers, because they are typically less willing to share the knowledge they have personally funded, than when they got their knowledge through public AES. All these are reasons why even in fully commercialized systems AES, state – public AES has important role. Only public service can guarantee coverage of all topics, even those where final beneficiaries are identified very broadly, which is especially important in the increasingly active role of AES in rural development. Also, public AES can provide greater coverage of farmers, including those who are not in economic position to use and pay private extension services.

The previous stated theoretical considerations are of particular importance for the planning of AES in Serbia. In fact, during 2009 and 2010 in Serbia was carried out a transformation of agricultural extension services from social enterprises to limited liability and joint stock companies, with a majority share of the state capital, and in 2010 a new Law on the performance of expert and extension services in the field of agriculture was adopted (“RS Official Gazette”, No. 30/10). In our country we still have mostly state-organized and financed AES.

Although the model of private financing of AES in Serbia is introduced in small steps, there are a number of users who need adequate services and who are in a position to self-finance them. According to Bogdanov (2007: 142-144), 24 percent of rural households in Serbia have a need for services that can be provided by the extension service (producers oriented on agricultural production are interested in information about the sources of credit, market, fertilization and protection of crops, while households that see their future outside of agriculture are interested in obtaining assistance in starting a new business). Unfortunately,

the model of agricultural extension services with selected farms applied in our country, which is the result of limited funding and modest human resources related to the number of individual farms, determined that according to the same survey 40 percent of farmers do not know that there agricultural extension service even exists.

Although this research, which included all rural households (agricultural, non-agricultural and mixed), was conducted before the reorganization of agricultural extension services in Serbia, this percentage is still worryingly high and indicates the need to focus more significant resources to finance agricultural extension services. A part of these funds could be provided with more allocations from the agricultural budget. According to the Regulation on the allocation of subsidies in agriculture and rural development (“RS Official Gazette”, No. 20/13), subsidies for agricultural extension service accounted for only 0.87 percent of the available funds. Allocations for AES for a long time have not been changed (in this year they are in absolute terms at the level of the average from the period 1997-2004 or about 2.3 million Euros), which is clearly not enough for a significant improvement or restructuring of this service. Increased participation of private organizations in the provision of extension services, which would primarily be focused on the larger farmers, would allow that extension workers employed in the public AES direct their activities to smaller farmers, which would at the national level increase the coverage of farms with extension work.

### **Conclusion**

There are two main sources of financing agricultural extension services: the public or state and private that can co-exist. Between these two extremes there are many intermediate forms and models of financing AES. Which financing model of AES will be best adapted and accepted in one country, depends on the characteristics of final users, their coverage – which is directly related to the topic that is addressed through extension services, the context and the framework of extension services and finally the organisation model of extension organization. Experiences from other countries can be of great importance for Serbia, because the commercialization of agricultural extension services is yet to come.

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## ANALYSIS OF PURCHASE AND PLACEMENT OF NON-WOOD FOREST PRODUCTS IN SOUTHERN SERBIA

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### Abstract

The long represented viewpoint that forests do not have economic value if it is not used for timber production, takes a new dimension. Namely, the more often mentioned so-called "other" forestry products and services have increasingly important role in market economies of many countries. In light of this, it is possible to single out non-wood forest products (NWFPs) which appear as a raw material for a range of finished products used in the pharmaceutical industry, food industry, etc. The area of Southern Serbia has a wealth of natural resources, but also a long tradition in the collection of wild medicinal herbs, berries and mushrooms. In this way, there is a real basis for the development of NWFPs sector in Southern Serbia. In order to gain insight into the dynamics of NWFPs commercialization in this area was carried out a survey of seven enterprises involved in their purchasing, processing and selling. The aim of the research is focused on monitoring the quantities of purchased and placed NWFPs in the period from 2004 to 2011. The purpose of the research is to indicate the potential for development of entrepreneurship based on NWFPs in Southern Serbia. The main research methods are modeling and trend analysis, while applied techniques are structured interview, SWOT analysis and its hybrid variant so called A'WOT analysis, which is a combination Analytic Hierarchy Process (AHP) and the classic SWOT analysis.

**Key words:** non-wood forest products, Southern Serbia, enterprises, trends, SWOT

### Introduction

Although there is no universally accepted definition of non-wood forest products (NWFPs), it is widely accepted view that these are all products from forests, other than wood (Ahenkan et al., 2011). Over time, NWFPs had different names, such as "minor", "secondary" and "small forest products". In many cases, these products are neither small nor minor and many of them have a long tradition of human use as well as timber products (Chamberlain et al., 1998). In recent years, domestic and international demand for NWFPs is growing and the forest sector is getting a new awareness of their importance. As a result, the NWFPs could be the main vehicle for economic growth, especially when there are no other resources (Greene et al., 2000).

Thanks to the variety of plant species and well natural predispositions, the area of Southern Serbia has been identified as highly suitable for the development of NWFPs based sector and organic production. However, in this region, despite the evident potential, are not sufficiently developed picking berries, herbs and other kinds of NWFPs. As a reason for this can be cited lack of awareness of the population about their commercial importance, methods of gathering them in nature, and processing technologies.

### Figure 1. Locations and number of interviewed enterprises

The study covered the period from 2004 to 2011. For this purpose managers of seven enterprises were interviewed in towns located in Southern Serbia: Vladi in Han, Surdulica, Crna Trava and Vranje (Figure1). The aim of the research was focused on examining the

quantities of NWFPs purchased and placed in the scope of the study sample of seven enterprises for a period from 2004 to 2011. The purpose of the research is, based on accurate data, to examine the possibilities for development of entrepreneurship based on NWFPs in the South Serbia region. It is focused on companies engaged in purchasing, processing and selling of NWFPs, purchased and marketed quantities and their prices on the domestic market.

### Material and methods

The study implements both general and special research methods. The primary methods were modelling, the trend analysis, and regression and correlation analyses. Long-term development trends, expressed as a function of time, called the secular trend or tendency (Stojkovic, 2001). In this paper, the trend is used to determine trends in the purchase and sale of certain types of NWFPs. To verify the obtained regression models are used the coefficient of determination ( $R^2$ ), the correlation coefficient ( $R$ ), t-statistics obtained from the parameters and F-statistics (Rankovic, 2009).

The paper used classical methods of reasoning: analysis, synthesis, induction and deduction. For the forming of the database is used a research technique such as structured interview. For the purpose of the research and data collecting a standardized questionnaire was created. Face to face interviews were conducted and in other cases questionnaires were sent by e-mail. From the questionnaire, for further analysis, were used those parts relating to information on quantities purchased and granted product and their selling prices. The other techniques that have been applied are SWOT and its hybrid variant, the so-called A'WOT, which is a combination of Analytic Hierarchy Process (AHP) and the classic SWOT analysis (Nedeljkovic et al., 2010). The basis for the application of AHP set by Thomas Saaty (Saaty, 1980) through idea and mathematical solutions. The application of this method was more in detail described by Jandric and Sr evic (2000). Over constructed SWOT matrix were identified strengths and weaknesses of interviewed enterprises, as well as external factors in the form of opportunities and threats. Through the A'WOT analysis, as synthesized form of SWOT and AHP, it is possible to form a hierarchy of priorities of elements included in the SWOT matrix and their mutual comparability (Kurttila et al., 2000; Pesonen et al., 2001).

The paper uses primary data and analyzes four major types of products that have had the biggest purchase and sale of the observed time interval. These are boletus, chanterelles, blueberries and raspberries.

### Results and discussion

All of interviewed enterprises are privately owned. All of them are mainly engaged in the purchase and sale of mushrooms and berries. Of all the products, for further analysis were extracted four that had the largest purchase and sale, such as boletus, chanterelles, blueberries and raspberries.

Unlike the purchase of raspberries and blueberries, which had a mild fall, purchase of boletus and chanterelles had a greater decline in purchase (Table 1). This can be attributed to lower yields in a given year and thus reduced demand and purchasing. Raspberries (117.6 t) and blueberries (110.3 t) had the highest average annual purchase.

**Table 1.** Structure and quantity of purchased raw products, for the period 2004-2011

Product	Quantity (in t)							
	2004	2005	2006	2007	2008	2009	2010	2011
Boletus ( <i>Boletus edulis</i> )	23.5	29.5	41.3	52.5	41.2	45	33.3	17.8
Chanterelles ( <i>Cantharellus cibarius</i> )	23.8	28.7	23.4	21.3	27.6	32.6	32.2	1.9
Blueberry ( <i>Vaccinium myrtillus</i> )	65	76	139	148	156	115	84	99.5
Raspberry ( <i>Rubus idaeus</i> )	90	92	154	156	144	136	86	83

Source: collected data

For trend analysis of all products, second degree polynomial trend is used. Purchase of boletus had a growing trend until 2007 followed by a period of stagnation until 2011 (Diagram 1). In the purchase of chanterelles it was found alternated sub-periods of growth and decline. In the last year of the observed interval, purchase of chanterelle drastically decreased as a result of weak yields in a given year (as well as boletus). Purchase of blueberries had a growing trend until 2008, followed by stagnation, with a slight increase in 2011. Purchase of raspberries had a growing trend until 2006 after which trend decreased.

**Diagram 1.** Purchase of NWFPs in the raw state for seven interviewed enterprises

All of the products have a positive growth rate, which is the most pronounced in the purchase of chanterelles and amounts to 18.9% (Table 2). Also, it was found that all of the products have a very strong correlation connection, except chanterelles whose correlation connection is medium  $R=0.435$ . By testing significance of the obtained correlation coefficients can be seen that all of statistical elements are significant at the significance level  $\alpha=0.05$ .

**Table 2.** Basic elements of regression analysis trend of purchased NWFPs in the raw state

Boletus $Y = -609236699 + 606969x - 151x^2$							
Parameter			t			R = 0.9074	F = 11.6497
a = -609236699	b = 606969	c = -151	t(a) = -4.82567	t(b) = 4.825739	t(c) = -4.82579	average exponential growth rate = 1.6%	
Chanterelles $Y = -121292205 + 120852x - 30x^2$							
Parameter			t			R = 0.4353	F = 0.5845
a = -12292206	b = 120852	c = -30	t(a) = -1.0596	t(b) = 1.0597	t(c) = -1.0598	average exponential growth rate = 18.9%	
Blueberry $Y = -52092932 + 518949x - 1292x^2$							
Parameter			t			R = 0.8238	F = 5.2799
a = -52092932	b = 518949	c = -1292	t(a) = -3.2188	t(b) = 3.21866	t(c) = -3.2184	average exponential growth rate = 3.5%	
Raspberry $Y = -554562438 + 552532x - 1376x^2$							
Parameter			t			R = 0.8775	F = 8.3664
a = -554562438	b = 552532	c = -1376	t(a) = -4.0485	t(b) = 4.04904	t(c) = -4.0493	average exponential growth rate = 1.6%	

Source: author's calculation

Enterprises perform purification of purchased raw materials and sell it as partially processed product. All of the products have a regressive movement to a greater or lesser intensity (Table 3). The largest average annual placement in the period from 2004 to 2011 had raspberries (90.2 t) and blueberries (77.6 t). More drastic drop in sales was recorded in the last year in placement of chanterelles and boletus (Table 3).

**Table 3.** Quantities and types of final products placed in the domestic market

Product	Quantity (in t)							
	2004	2005	2006	2007	2008	2009	2010	2011
Boletus (Boletus edulis)	18.5	23.5	37.2	47	30.7	40.6	31.1	14
Chanterelles (Cantharellus cibarius)	22.4	27.3	22	20	26.3	31.3	31	1.5
Blueberry (Vaccinium myrtillus)	45	55	93	112	105	95	56	61
Raspberry (Rubus idaeus)	65	65	125	125	113	105	61	62.5

Source: collected data

For trend analysis of all products, second degree polynomial trend is used. For all of products stagnation was recorded especially in the last years of the period (Diagram 2).

**Diagram 2.** Placement of NWFPs for seven interviewed enterprises

All of the products have a positive growth rate, which is the most pronounced in the purchase of chanterelles and amounts to 22% (Table 4). Also, it was found that all of the products have a very strong correlation connection, except chanterelles whose correlation connection is medium ( $R=0.377$ ) and the parameters are not statistically significant; due to that results related to the placement of chanterelles should be taken with caution.

**Table 4.** Basic elements of regression analysis trend of products placed on the domestic market

Boletus $Y = -469389947 + 467631x - 116x^2$				
Parameter		t	R = 0.8306	F = 5.5627

$a = -469389947$	$b = 467631$	$c = -116$	$t(a) = -3.3341$	$t(b) = 3.3340$	$t(c) = -3.3340$	average exponential growth rate = <b>0.8%</b>	
<b>Chanterelles <math>Y = -95120645 + 94812 \cdot x - 23 \cdot x^2</math></b>							
<i>Parameter</i>			<i>t</i>			<b>R = 0.3776</b>	<b>F = 0.4157</b>
$a = -95120645$	$b = 94812$	$c = -23$	$t(a) = -0.6523$	$t(b) = 0.65262$	$t(c) = -0.6529$	average exponential growth rate = <b>22.0%</b>	
<b>Blueberry <math>Y = -298645284 + 297516 \cdot x - 740 \cdot x^2</math></b>							
<i>Parameter</i>			<i>t</i>			<b>R = 0.8846</b>	<b>F = 8.9962</b>
$a = -298645284$	$b = 297516$	$c = -740$	$t(a) = -4.2224$	$t(b) = 4.2222$	$t(c) = -4.2220$	average exponential growth rate = <b>2.6%</b>	
<b>Raspberry <math>Y = -376698347 + 375318 \cdot x - 934 \cdot x^2</math></b>							
<i>Parameter</i>			<i>t</i>			<b>R = 0.8533</b>	<b>F = 6.6938</b>
$a = -376698347$	$b = 375318$	$c = -934$	$t(a) = -3.6248$	$t(b) = 3.6251$	$t(c) = -3.6253$	average exponential growth rate = <b>1.4%</b>	

Source: author's calculation

Wealth of natural resources and the availability of raw materials, the production of high quality products and competitive prices are the most significant strengths of interviewed enterprises (Table 5). Weaknesses are the lack of processing facilities and outdated equipment with non-specialized manpower and products features (perishable products). Opportunities are manifested through the growing demand for the products of natural origin and orientation to organic production. In addition, in order to encourage the export of these products, opportunities could be forming association of small entrepreneurs and production in accordance with the standards. The threats are underdevelopment of domestic markets for NWFPs, variability of yields in nature which influence the volume of purchase and placement, as well as the market prices in a given year. Also, it is necessary to point out to the competition and the current economic crisis.

**Table 5.** SWOT analysis

<b>S (strengths)</b> s1 - natural resources s2 - availability of raw materials s3 - products quality s4 - price competitiveness	<b>W (weakness)</b> w1 - lack of processing facilities w2 - non-specialized workforce w3 - outdated equipment w4 - product features	<b>O (opportunities)</b> o1 - growing demand o2 - association of small entrepreneurs o3 - organic production o4 - quality and standardized products	<b>T (threats)</b> t1 - undeveloped market t2 - instability of plant yield t3 - unfair competition t4 - economic crisis
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Source: by author

On the diagram 3, it is shown the extent to which chance, compared to the other elements of SWOT analysis are extracted, which confirms their greatest distance from the coordinate centre. The most valued elements in the SWOT matrix are opportunities, with a value of 0.304 suggesting the existence of preconditions and opportunities for the development of NWFPs sector in the South Serbia (Diagram 3). The most important elements are the growing global demand for the products of natural origin (o1) and forming the association of small entrepreneurs with the aim of encouraging exports primarily to the wider regional and whole European markets (o2) (Diagram 4).

**Diagrams 3 and 4.** A'WOT analysis



Compared to wood, the use of NWFPs is possible without major damage to the forest and its environmental impacts (Ros-Tonen, 2003). Southern Serbia is a mountainous region, with an economy based on natural resources which opens the possibility to develop entrepreneurship based on NWFPs. The lack of processing capacity of enterprises greatly reduces the economic impact that can be achieved by increasing degree of product finalization ( a et al., 2012 ). In the case of the interviewed enterprises there is a lack of processing capacity, because of the products are sold in the unprocessed state. Also, there is a lack of marketing activity, in order to successfully implement the commercialization of these products groups ( a et al., 2012b).

Based on research conducted in Switzerland it was found that in the second place of demand of NWFPs are mushrooms and berries (Seeland et al., 2007). The interviewed enterprises mostly purchase and sell these two types of products. However, there are not enough marketing activities and state support that would contribute to the development of a sector based on NWFPs.

The current prices of these products are: boletus 2.3 €kg<sup>-1</sup>, chanterelles 5.9 €kg<sup>-1</sup>, blueberry 1.8 €kg<sup>-1</sup> and raspberries 1.5 €kg<sup>-1</sup>. Prices depend on the relationship of supply and demand, operating costs, and the current amount of raw material on the market. The interviewed enterprises price products by the method of "cost-plus", where the starting point is to cover the cost with a certain percentage of margin. All of the interviewed enterprises are confident in their price competitiveness in the domestic market. Competitive prices are achieved by low labor costs and availability of raw materials. While producers have access to markets, their most important challenge is to increase the quality and quantity of production at competitive prices (Belcher et al., 2007). However, it is necessary to organize staff training in order to better productivity and quality of work (Nedeljkovic et al., 2010). Relatively low labour costs represent a comparative advantage for Serbia in relation to the EU markets and others international markets too (Salmi et al., 2006). The interviewed enterprises purchase and sell products, which are classified as typical NWFPs. All of interviewed enterprises purchase NWFPs in their raw state and perform their resale as unprocessed products. Products are sold on the domestic market and not exported yet. Because of it, is necessary the support and encouragement of state institutions for the development of innovative activities of entrepreneurs and providing support for product placement outside of Serbia. In order to increase profits it should be developed greater range of products and produced final products with high level of finalization, as well as investing in the "on-line" sale. Systems such as "quick" sales or e-commerce have a great importance for all categories of products, especially for perishable products (Pettenella et al., 2006).

### **Conclusion**

By analyzing data obtained by interviewing seven enterprises can be formulated the following conclusions:

- the highest increase in purchases had chanterelle (18.9%) during the period from 2004 to 2011, while the purchase of other products was lower: boletus 1.6%, blueberries 3.5% and raspberries 1.6%;
- the highest growth was achieved in the sale of chanterelles with growth of 22% and significantly lower growth in sales of boletus 0.8%, blueberries 2.6% and raspberry 1.4%;
- enterprises are not export-oriented;
- the highest average annual purchase had raspberries (117.6 t) and blueberries (110.3 t);
- the largest average annual placement had raspberries (90.2 t) and blueberries (77.6 t);

- the highest current price of 5.85 €kg<sup>-1</sup> has chanterelle, then boletus 2.25 €kg<sup>-1</sup>, blueberry 1.80 €kg<sup>-1</sup> and raspberry 1.49 €kg<sup>-1</sup>;
- strengths of the interviewed enterprises are: wealth of natural resources, availability of raw materials, production of high quality and price competitiveness;
- their weaknesses are: outdated processing facilities and equipment, with a non-specialized labor and products which are categorized as "perishable products";
- opportunities are manifested through the growing demand for the products of natural origin, formation of associations of small entrepreneurs and production in accordance with the standards. Opportunities are, according to the results of A'WOT analysis, the most valued element with a value of 0.304;
- threats are: insufficient development of domestic markets for NWFPs, variability of yields in nature which influence the volume of purchase and placement, as well as market price in a given year. Also, it is necessary to point out to the competition and current economic crisis.

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**PROBLEMS IN RECORD KEEPING OF PRODUCTION AND ECONOMIC RESULTS OF FAMILY FARMS IN THE REPUBLIC OF SERBIA**

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**Abstract**

In order that agriculture, one of the most important branches of the Serbian economy, can function satisfactorily, appropriate agrarian policies need to be implemented. When setting up agrarian policy measures, it is necessary to have a good information base not only for agricultural economic analysis as a basis for creating appropriate measures, but also for monitoring and evaluation of the implemented measures. Systematic record keeping on family farms is a basis for creating an appropriate agricultural policy in the domain of family farming, as well as for conducting further analysis that can contribute to the successful implementation of the agricultural policy. Looking at the previous developmental period, it can be concluded that most of the problems occurred due to the lack of adequate economic data and indicators of the business of some family farms that have a large share in Serbian agricultural resources and contribute significantly to production of many agricultural products. In line with the reforms undertaken in all industries, and in order to harmonize laws and regulations with those of the EU in the process of EU accession, changes have also been occurred in Serbian agriculture, starting from introduction of systematic record keeping of production and economic performances on selected family farms, which will help in monitoring of basic indicators necessary for proper adoption of agrarian policy measures.

This paper describes the system of record keeping of production and economic business indicators on family farms that has been applied in the EU countries, and has now being introduced in the Republic of Serbia. The paper also gives a review of the dynamics of its introduction together with the analysis of the recently achieved results.

**Key words:** business records, information base, family farms, agricultural policy, Republic of Serbia.

**Introduction**

Family farms represent the smallest agricultural units of a country. This, however, does not diminish their importance. An appropriate agrarian policy aimed at this smallest unit results in strengthening the whole system. In the Republic of Serbia, family farms are particularly important units of the agricultural system, since they have a large share in Serbian agricultural resources and contribute significantly to production of agricultural products.

Besides playing a role in feeding population, agriculture also provides inputs for many industries. It also uses a large number of inputs from other industries, therefore representing a significant market for a series of industrial products (Vasiljevic & Zakic, 2008).

Agriculture is characterized by many specificities, the most important of which originated from its three basic characteristics:

1. Agriculture represents the production of organic matter which, apart from human labor, mainly depends on environmental conditions (climate, landscape, geographical position and soil fertility);
2. Special resources are used in agricultural production, such as land, animals and perennial plantations;
3. Agricultural production includes many different subjects, such as family farms, large agricultural corporations and cooperatives – special organizations that combine an association with a business company. (Vasiljevic, 2012)

Transitional period and the process of EU accession have brought a lot of changes in Serbian agriculture, making its subjects accept those changes and the process of adapting to standards that are applied in the EU and developed countries. Beside introducing new technologies and putting more emphasis on innovations, these processes imply the necessity of establishing record keeping on family farms in order to monitor costs and production results systematically and to create an adequate basis for implementation of agrarian policies.

In the long run, it is necessary to manage the development of family farms on a strategic level. To accomplish that, some quality and valid information on the scope and structure of agricultural production is needed, as well as information on economic effects. In that respect, a necessary measure is the establishment of accountancy and information system to record business changes on farms and to compile them on a national level. (Pejanovic, 2007)

### **Material and Methods**

In this paper, research methods and procedures specific to the economy and agricultural economy were used.<sup>20</sup> This paper combines different research methods. The analytic and synthetic research methods for the subject matter were used, as well as so-called “desk analysis” of references and obtained results. The comparative analysis was used for comparing the obtained results in Serbia with the ones in European countries. The paper analyses the main characteristics of the *FARM ACCOUNTANCY DATA NETWORK* (FADN) methodology used for recording and processing data collected on the family farms chosen in the sample.

### **Results and Discussion**

Developed countries recognized the need for farm record keeping long time ago. Although it was known in the late 18<sup>th</sup> century, record keeping in Germany became mandatory in 1953, while its expansion occurred in the late 19<sup>th</sup> century. In 1925 France founded its first institution for record keeping, and one more after the World War II. In Italy, a simple accountancy system was introduced in 1926.<sup>21</sup>

In Serbia, the first research on accountancy dated before the World War II, in 1921. The Faculty of Agriculture and Forestry was in charge of this research, and the first funding for it was approved in 1938. Unfortunately, the data on the research conducted until 1941 on 900 farms in 44 villages were burnt down by the German army just before the liberation of the city of Belgrade.

After the World War II, in 1949 the government of the Federal National Republic of Yugoslavia (FNRY) put in charge the Institute of Agricultural Economics from Belgrade to

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<sup>21</sup> Krstic, B., Jevtic, S., Arsenovic, DJ. (2005): Knjigovodstvo na seljackom gazdinstvu, IPN, Beograd, pp. 4-5.

collect data on farms. Thousands of farms were surveyed in that period, and the focus was put on taxing of farmers and the analysis of costs and prices of agricultural products. Although the obtained results were there for implementing agrarian policies and writing master and PhD theses, this research was stopped in 1981 due to the lack of funding. In the next two decades there were no valid farm data until 2001 when implementing the “Monitoring selected registered farms” project, funded by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia and conducted by the Institute for Science Application in Agriculture (IPN) from Belgrade, resulted in the first relevant data on family farms. That year, data on 1774 individual farms were taken into the sample.

Due to political changes and other issues this research was not conducted in 2005 and 2006, and it continued in 2008<sup>22</sup>. Nowadays, the IPN monitors 3550 selected leader farms through 22 regional agricultural extension services of the Republic of Serbia (PSSS) (Table 1).

Table 1 Number of selected leader farms in Serbia monitored and given by the regions (2013)

No.	PSSS	Number of extension agents	Number of municipalities	Selected leader farms	Number of farms
1	Sabac	9	5	25	225
2	Loznica	5	4	25	125
3	Valjevo	8	6	25	200
4	Smederevo	7	4	25	175
5	Pozarevac	8	8	25	200
6	Kragujevac	8	7	25	200
7	Jagodina	7	6	25	175
8	Negotin	7	4	25	175
9	Zajecar	6	4	25	150
10	Uzice	7	8	25	175
11	Cacak	8	4	25	200
12	Kraljevo	6	4	25	150
13	Krusevac	9	6	25	225
14	Nis	6	7	25	150
15	Prokuplje	6	4	25	150
16	Pirot	5	4	25	125
17	Leskovac	8	6	25	200
18	Vranje	5	7	25	125
19	K. Mitrovica	3	7	25	75
20	P. Skela	4	4	25	100
21	Mladenovac	6	7	25	150
22	Novi Pazar	4	3	25	100
<b>Total</b>		<b>142</b>	<b>119</b>	<b>3550</b>	<b>3550</b>

Source: Report on extension activities conducted by the agricultural extension services in the first quarter of 2013, submitted to the Ministry of Agriculture, Forestry and Water Management by the IPN

<sup>22</sup> Vasiljevic, Z., Sevarlic, M., Filipovic, N., Rahovic, D. (2008): Uvodjenje knjigovodstva na porodica gazdinstva u Srbiji, Zbornik „Evropska unija i zapadni Balkan – Izazovi za agrarnu privredu Srbije: sta nam je ciniti“, Regionalna privredna komora – DAES, Novi Sad – Beograd, pp. 57-61.

Extension agents are obliged to fill in the survey sheets for gross margins for dominant crops on 3550 leader farms. They collect data on their leader farms and send those data to the IPN at the end of each production year.

„According to the Annual work plan, every extension agent should calculate gross margins based on the parameters they record for each chosen farm. Every extension agent should calculate 25 gross margins for their leader farms for dominant enterprises (one gross margin per farm). By the end of the first quarter of 2013, extension agents submitted a total of 3575 gross margins collected during 2012. Most of the gross margins were for crop and vegetable production (1499), then for fruit production and viticulture (1067) and for animal husbandry (997). As for agricultural processing, 11 gross margins were collected, and one gross margin was made for services“<sup>23</sup>

Considering that for a long time there was no record keeping of costs, this relatively small sample can give certain indicators on costs and economic results on farms (that are otherwise not obliged to do the record keeping), which is surely important both for choosing more appropriate measures and determining the direction of the agrarian policy.

Simultaneously with the record keeping on gross margins for dominant enterprises of selected leader farms done by the IPN, in October 2011 the long-awaited FADN project (*Farm Accountancy Data Network*) was officially launched to establish a network for collecting data according to the FADN methodology. Due to this methodology, the physical and economic data from the sample of selected leader farms in Serbia would be systematically collected in the same way this is done in the EU countries.

The FADN system or “Farm Accountancy Data Network” has proved its efficiency in the European Union. The system was established to enable microeconomic analyses and determine financial position of a farm on a macroeconomic level, and it is fully supported by the EUROSTAT. The FADN concept was established in 1965, when a regulation known as *the Council Regulation 76/65* determined a legal basis for this network organization. This concept is based on annual collection of production, economic and financial data from a sample of farms divided into groups according to the following three criteria: economic size of farms, type of production, and regional position. Conducting annual research is an obligation for all EU member countries, and it represents a tool the European Commission uses for assessing farm income and identifying the effects the *Common Agricultural Policy – CAP* has on farm business<sup>24</sup>.

The established and operating FADN system is one of the perquisites Serbia has to fulfill in the process of EU accession.

Like all other projects on record keeping, this project is also facing big problems. One of the basic problems is how to include an adequate number of farmers, considering that it is based on voluntarily participation in the network and having in mind well-known farmers’ lack of trust. Moreover, one of the problems is also that farm owners do not believe strongly enough that this is beneficial to their farms.

Rural depopulation, a great problem of the whole agro-industry, also implies the lacking of the person who will inherit the farm, which is the problem the implementers of the project on establishing the system for monitoring and record keeping of farm data are facing. In a broader sense, this problem also tackles the farm ownership structure. It is questionable how efficient record keeping can be for small farms, since the average farm size in Serbia is about 3 ha.

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<sup>23</sup> Report on extension activities conducted by the agricultural extension services in the first quarter of 2013, submitted to the Ministry of Agriculture, Forestry and Water Management by the IPN, pp. 12.

<sup>24</sup> Vasiljevic, Z. (2012): Uspostavljanje mreze racunovodstvenih podataka na porodicnim poljoprivrednim gazdinstvima u Srbiji, *Casopis za pravo i ekonomiju evropskih integracija – Izazovi evropskih integracija*, Beograd, pp. 34.

The problem of the farm ownership structure also affects farms that have an heir but do not own the land – they lease it. Moreover, farmers do not often report on the whole land they farm, since they do not have a lease agreement. The land owners often give their land for leasing without making a lease agreement, yet being subsidized for their crop production by the government. This increases the costs “real farmers” have and diminishes their competitiveness.

In order to improve the competitiveness of their production, a number of advanced farmers have identified a need for improving the knowledge, so they voluntarily decided to participate in such projects, like the one on the establishment of the FADN. These projects help them monitor their own farm business, measure and diminish the costs through applying and recording adequate cropping practices in order to have better insight in farm cash flow, therefore improving the business of farms that joined the project.

Adopting a law on mandatory farm record keeping would ease this process significantly. Therefore, data for projects like the FADN would be more relevant, enabling the government to have more relevant information for improving measures of agrarian policies.

Making farm record keeping mandatory will not necessarily solve the problems of rural depopulation; improve the farm ownership structure, solve the problem of farmers’ lack of trust, etc. This measure, however, would certainly help in diminishing production costs, making more appropriate decisions on investments in more profitable and sustainable production, and making agricultural production more specialized.

### **Conclusion**

Observing the genesis of the practice of recording and monitoring the economic and financial indicators of family farm business in Serbia, as well as periods when no records were kept, it can be concluded that the existence of a permanent and systematic record keeping of costs and business results is an important factor for the creation of appropriate measures of agrarian policy, and improving competitiveness through reducing production costs. By improving agricultural production and harmonizing national legislation with the EU legislation through the creation of an appropriate information system for data collecting, processing, analyzing and storing, some requirements set out in the IPA 2010 programming cycle are also fulfilled. Agrarian policy cannot be adequately managed without having relevant data and records, while incentives (subsidies) allocated for specific purposes also cannot be allocated to certain production enterprises and measures, if not based on adequate data and analyses.

One of the outcomes of the systematic monitoring of farm economic results is reflected in an adequate insight into farm cash flows, which can help in establishing cooperatives and associations. These cooperatives and associations through machinery rings could reduce production costs, increase profits and ensure the competitiveness of products from family farms in Serbia.

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**CONTRIBUTION OF VIRTUAL WATER TO IMPROVING WATER SECURITY IN  
TUNISIA: A CASE STUDY OF WHEAT AND OLIVE GROWING FARMS IN  
ZAGHOUAN REGION**

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**Abstract**

Virtual water represents all freshwater used in the process of producing a commodity. In the case of agricultural products, many studies have focused on quantifying virtual water flows through international trade products. The concept of virtual water commercialization should be carefully studied as a potential solution for water scarcity, especially in countries facing risks of water shortage in a few years such as in Tunisia.

The main idea of this paper is to optimize water use, by the mean of estimation of virtual water in exported crops which have high water consumption. We also analyze the crops that are imported and therefore, might contribute to save water.

Commonly exported and imported crops are widely cultivated in the region of Zaghouan characterized by diversity of agricultural products. That's why it could represent a good case study from Tunisia. In this study we especially focus on olive oil which is one of the most strategic exported products in Tunisia and on wheat as main imported product.

We attempt to create technical and economic data sheets through monitoring about 40 farmers in this region. These sheets are not only to estimate the gross margin but also to calculate water demand for each crop allowing the estimation of virtual water. We found out that Tunisia may saves 1.13 m<sup>3</sup> of water per kilogram of wheat if we import it instead of producing it domestically. In the case of olive trees, for an average yield of 2339 kg per hectare we exports 2.10 m<sup>3</sup> of virtual water for every kilogram of exported olive.

Results presented in this study are of essential implications for policy making regarding water use optimization and water security enhancement.

**Keywords:** Tunisia, water scarcity, virtual water, international trade, water security.

**Introduction**

Agriculture has always occupied an important place in the socio-economic development in Tunisia. The agriculture expansion is heavily relied on available natural resources, especially on water. Because of its geographical location, Tunisia undergoes the influence of two climate types: the Mediterranean type in the north and the Saharan type in the south which are at the origin of space and time variability in water resources. Therefore, the annual rainfall average varies from less than 100 mm in the extreme south to more than 1500 mm in the extreme northern parts of the country. Water resources are evaluated in 2000 to 4825 million m<sup>3</sup>, with 2700 million m<sup>3</sup> of surface water and 2,125 million m<sup>3</sup> of ground water. Tunisia is, then, a country with relatively limited renewable water resources (SEMIDE, 2002).

That's why; taking into consideration the scarcity of water resources in the planning of agricultural policies is necessary to improve the trade balance, to ensure a level of food security, and to enhance the countryside and the environment.

In this context, many countries attempt to reach new alternatives for the management and the sustainable use of water resources in particular. These new approaches have brought about a relatively new idea called virtual water (VW) which tries to give an explanation to water use management (Velázquez, 2007).

### Virtual Water Concept

The Concept of virtual water was firstly defined by Allan (1993, 1994) in terms of the water embodied in a certain product, that is, the water necessary for creating a product.

In food products or crops products trading, there is a virtual water flow between producers and exporting countries and countries that consume and import those goods. Zimmer and Renault, (2003) had concluded that it did not make much sense to export goods that required a lot of water in countries with water deficits.

Therefore, countries facing water shortages should tend to import goods whose production requires a lot of water rather than produce these goods. The transaction resulted in actual water savings; it allows the country to not only ease the pressure on water resources, but also to save its available resources to use for other purposes.

According to the Blue Plan (2008), agricultural products' trading is responsible for nearly 90% of virtual water exchange in the world. It is indeed difficult to carry water, then it is easier to trade agricultural products whose production is a major consumer of water in the world (Fernandez and Thivet, 2008). Thus, food import is equivalent to the import of water condensed form. This is called virtual water import (Allan, 1998).

The virtual water concept has also shown up influence of consumer's food preferences and agricultural policies in the surplus producing areas to the detriment of water resources (Renault, 2002) and consequently has drawn attention internationally to the issue of the irrigated agricultural activity distribution between different parts of the world and the organization of global food markets.

Quantification of virtual water flows in agriculture allows illustrating interactions between agricultural and water policies and their impact on the use of water resources. Policies to reduce the export of products whose production requires large amounts of water are occasionally adopted by many countries in situation of water shortage. These also tend to increase their imports of agricultural commodities.

Particularly Middle East and North Africa countries, potentially illustrate a virtual water trade strategy to compensate for water shortages and to enhance their food security (Wichelns, 2001).

To ensure food security, Tunisia imports a lot of agricultural products such as wheat, corn and barley. In 2011, wheat imports reached almost 2 million tons (FAOSTAT, 2011). In addition to food security, these imports can ensure better conservation of water resources. Such a strategy is efficient during years in which the world price of grain products is less than the cost of production especially in countries, like Tunisia, with a potential water scarcity. Nevertheless, the export of some food products such as olive oil, citrus, dates, etc., even if it involves the export of huge amounts of virtual water, it remains vital to Tunisian economy. Nearly three-quarters of these products are intended for the European market.

### Objectives and structure of this study

To set this work in the right context, it is important to note that is part of a larger study in the framework of a research project about “virtual water and food security in Tunisia: from observation to support to development”. This project aims, mainly, to assess the potential and challenges of virtual water in food security strategy in Tunisia and its implications on the economy of water resources.

Regarding this work, goals are more specific. Based on gross margin collected data sheets we will try to assess value of each m<sup>3</sup> of water consumed by each studied culture. Next, we will focus on the estimation of virtual water amount needed to produce two strategic crops in Tunisia; Olive growing, since olive oil is one of the most important exported agro-food products with about 100 thousand tons in 2010 and wheat as the most imported product with 1 million tons in 2010 (INS, 2010).

Field work took place in the region of Zaghuan located in the north-east of Tunisia. The choice of this area can be justified by the fact that agricultural activities are focused on cereals and olive growing. Agricultural land is covering two thirds of the region territory with 282,000 ha, (185,000 of arable land and 87 thousand of range and forest) and water resources are mobilized by two large dams and 18 small lakes. The agricultural sector contributes significantly to regional economic growth especially since it provides about 27% of jobs.

This work would provide the basis for any discussion about Tunisian agricultural policies, rational and efficient water management in the field of agricultural production, water resources conservation and food security. The study tries to go one step forward in this field by breaking fresh ground for future research. The paper is organized as follows. After the introduction, we will explain the methodology we have used to create technical and economical data sheet, calculate gross margin and estimate virtual water. Results are analyzed in part 3. Final remarks and conclusion are included in part 4.

### Materials and methods

In this work we have been monitoring the management of 22 wheat farmers and 13 olive trees farmers during a full crop season to create technical and economic data sheets, to calculate gross margins and estimate the virtual water for each studied crop. The survey covered various parts such as farmers’ identification, acreage, mechanization, labor, seeds, irrigation, fertilization, treatments, yield and selling prices. All questions were about quantities, prices, dates of farming activities, costs and incomes.

Obtained information is used to calculate charges and gains of each farmer and to deduce gross margin.

$$\text{Gross margin} = \text{Total incomes} - \text{Total variable charges}$$

Technical and economic data sheets have been developed for each farm then weighted average sheets have been made for each crop. The weighting is based on the farm size.

After that, we attempt to estimate virtual water (m<sup>3</sup>/kg) for wheat as an imported product and olive oil as an exported one. As we saw previously, virtual water is the water embodied in products, in other words, the water which is necessary to produce certain goods. Hoekstra (2002, 2003) suggested two branches to the concept to analyze the amount of water that can be saved if we import goods instead of producing them domestically: real virtual water and theoretical virtual water. The first involves the water that is actually used in the domestic production of goods. In turn, the second is the water that would have been used in the country of destination for a same production. In order to estimate the virtual water associated to olive

oil, we will estimate the amount of water actually used to produce olive oil in the studied zone. Actually we will use the concept of real virtual water created by Hoekstra et al. (2003). Conversely, we will employ the concept of theoretical virtual water to estimate the virtual water imported in wheat crops. This means the amount of water that we would have needed if we have not imported wheat but produced it instead. This theoretical concept is more suitable than the concept of real virtual water to estimate imports of virtual water in this case. This amount of water does not have to coincide with the amount we would be using in country we are importing from, on account of the differences in weather conditions, soil texture, evapotranspiration, etc. (Velázquez, 2007).

The first step to calculate virtual water is to estimate the Water Needs of a harvested Crop  $n$  (WNC $n$ ), expressed in  $m^3/t$  (Chatzimpiros and Barles, 2007).

$$WNCn = ETPn / Rn$$

ETP $n$  is potential evapotranspiration of the plant (mm / period of growth, calculated by the Penman-Monteith method (Allen et al., 1998) and made available by the FAO (Food and Agriculture Organization of the United Nations) and R $n$  the  $n$  crop yield (t / ha) (Agreste, 2006). The potential amount of water (PW $n$ ) per crop per year is then calculated by multiplying the harvested production (P $n$ ) by WNC $n$ .

$$PWn (m^3) = WNCn (m^3 / t) * Pn (t)$$

Calculation of water productivity is rather simple: crop water requirements ET $a$  ( $m^3/ha$ ) are calculated from the climatic demand (ETP) adjusted with crop coefficients (K $c$ ). Software like CROPWAT (FAO, 1992) can be used for this purpose. Water productivity is then obtained by dividing the crop yield Y (kg/ha) by the crop water requirements. Virtual water value (VWV), the inverse of water productivity is then given by the following equation:

$$VWV = Et_a / Y$$

So, virtual water of the crop per unit ( $m^3/kg$ ) has been estimated as the ratio of the water consumption ( $m^3/ha$ ) to the crop yield (kg/ha). The water consumption is obtained by summing up the real water evapotranspiration over the growing period of rainfall and irrigated systems. The water consumption includes the proportion of the water evapotranspiration of each textural class. The virtual water volume in  $m^3$  is calculated multiplying the final water consumption over the growing period and the crop area. The inclusion of water consumption depending on the textural class is an improvement of the method of Hoekstra et al. (2009) made by Salmoral et al. (2010).

Calculations were made with an Excel application elaborated by the project team and checked by the CROPWAT software.

### Results and discussion

Conducted field surveys showed us that Tunisian farmers are usually unaware of the water needs of crops. Irrigations doses are made most of the time by the amount of available water and archaic skills based on traditional tools (foliage observation, assessing soil moisture, etc.). Distribution of irrigation doses is set randomly without really recognizing critical stages of plants development. This can be explained by the low level of education of the majority of the interviewed farmers (more than 60%) and their age that usually exceeds 50 years. The age and educational levels of the sample match with national statistics. In fact, according to the survey on the agricultural exploitations structure (2004-2005) carried out by the Ministry of

Agriculture, Water Resources and Fisheries, about 70% of Tunisian farmers are aged over 50, illiterate or with primary school education.

Concerning crops, we can say that average weighted data sheet obtained for wheat farming in the region of Zaghouan shows that for an average yield of 60 quintals/ha, gross margin is about 500 € The value of one m<sup>3</sup> of irrigation water is estimated at 0.170 € As for the olive, despite it is a low cost production crop, the average gross margin does not generally exceed 500 € The olive trees need supplementary irrigation only during the dry period in the studied zone. One m<sup>3</sup> of water used for the irrigation of olive trees leads approximately to a gross margin of 0.5 € These results are confirmed by the study of Bachta et al. (2001) about competitiveness of exported Tunisian agricultural products and costs in natural resources. This work shows that olive oil exported by Tunisia has a statistically significant explanatory power of competitive performance, contrary to wheat.

Virtual water comprises three components of different colors: the green, blue and grey waters (Allan, 1998). Quantification of virtual water flows for each of the two studied cultures showed that for wheat, volume of virtual water used is equal to 682.5mm which is the sum of the green water (useful rain) with 292 mm and blue water (irrigation water) with 390.4mm.

$$VW = VW1 * 10 / Y = 6825/6018 = 1.13 \text{ m}^3/\text{kg}$$

VW: Virtual Water expressed in m<sup>3</sup>/kg

VW1: Virtual Water expressed in mm

Y: Yield in kg/ha

In other words Tunisia saves 1.13 m<sup>3</sup> of water per kilogram if we import wheat instead of producing it in the region of Zaghouan. In the case of olive trees, the amount of virtual water used is equal to 493.5 mm or (408.5 mm, 85mm green water and blue water). This means that for an average yield of 2339 kg per hectare, virtual water is about 2.10 m<sup>3</sup>/ kg. Consequently, Tunisia exports 2.10 m<sup>3</sup> of water for every kilogram of exported olive. This amount will increase considerably when we involve olive oil processing water consumption.

Importing significant quantities of wheat represent a good strategy especially when the world price is below the cost of local production. As we have already seen, by importing about 1 million tons of wheat per year, Tunisia is saving a huge amount of virtual water. Indeed, wheat is a vital commodity for food security in water scarce importing countries (Yang et al., 2006). It is also important to preserve local production. That's why; wheat production is still being closely related to agricultural and water policy of the country.

Finally, although farming products' needs of virtual water amount vary according to crops, varieties themselves, crops managements, etc., the encountered results are comparatively close to those reported by Zimmer and Renault (2003).

### Conclusion

Through the two studied crops examples, we intend to contribute to the achievement of a more efficient allocation of water resources, and to making a first step towards a comprehensive analysis of two dimensions of virtual water - agronomic and economic - in a perspective of sustainable development and improved food security.

The challenge is to develop the country's capacity to produce crops while preserving and enhancing its limited water resources through the choice of crops that use less water and guarantees good returns.

Indeed, in the current context of scarce water resources, farmers should be better encouraged to rationalize water use. Managers should be able to ensure an equitable distribution of available resources based on crop needs. Therefore, we can say that the opportunities to improve water efficiency in the agro-economic level is based primarily on a better planning of land use and a proper management of the limited water resources. Valuing virtual water is also dependent on the establishment of a proper irrigation schedule that provides the plant with its water needs in its vegetative stages. Irrigation system must be revised to increase its efficiency. All that can have a positive impact on limiting water wastage.

Tunisia exports water-extensive high-value economic products adapted to the Mediterranean climate, essentially olive oil. It is true that by exporting olive oil Tunisia loses a significant amount of virtual water, but these exports are very important from an economic and social point of view.

Finally, it is important to say that further analysis will be carried out in the project “virtual water and food security: from observation to support to development”. These studies could provide a transparent and multidisciplinary framework for informing and optimizing water policy decisions, contributing at the same time to the implementation of a national water directive.

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**COST, RETURN ANALYSIS AND CONSTRAINTS IN LIVESTOCK PRODUCTION  
AND MARKETING IN HAI DUONG, VIETNAM**

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**Abstract**

The study, using the survey data from 145 livestock production households, showed that livestock contributed significant parts to the households' income. Given a production unit, the high investment in inputs and the considerable experience in production and marketing created higher income for the livestock-based group than that for the non livestock-based group. However, the farmers perceived some constraints relating to both production and marketing. The result from the Garrett's ranking technique presented the ranking position of constraints, respectively included the livestock disease, the limited credit access, the high and rapid increase in feed price, the high volatility of output price, and the insufficiency of market information and weak bargaining power.

**Key words:** Livestock, Constraints, Garrett's ranking technique.

**Introduction**

Livestock production is predominantly operated in small-scale production units. Presently, the small producers supply the majority of meat in the market. About 80% of poor households in Vietnam raise livestock and 30% of total agricultural income of households is generated from livestock production (Lapar et al., 2003). Hai Duong has the potential for livestock production as it is located near Hanoi capital, where the demand for meat and fish by consumers has gradually increased. The agricultural labor currently accounts for 64.2% of the total labor in Hai Duong (GSO, 2012). Livestock production not only generates more income for farmers but also reduces the migration flow from the rural area to the urban area. However, recently livestock producers have confronted with some unfavorable factors (DARDHD, 2010). With the important role in livestock production, the improvement in livestock production and marketing is very crucial to create a stable income for farmers. The paper is to analyze cost and return of livestock production, and to explore some main constraints related to production and marketing by the small livestock producers.

**Material and methods**

✓ **Data collection:** The primary data were collected from the household survey, which was made using both stratified and random selection. 145 farm households engaged in livestock production were selected for data collection.

✓ **Data analysis**

SPSS software was used for data processing using descriptive statistics and analysis of variance. In addition, the Garret's ranking technique was employed for the relative assessment of constraints associated with livestock production and marketing. The respondents were asked to rank their constraints. The individual's ranking was converted into percentage position for each of the assigned ranks by using the formula given below (Garret and Woodworth, 1971).

$$\text{Percent position} = \frac{100 (R_{ij} - 0.5)}{N}$$

where:  $R_{ij}$  = Rank assigned for the  $i$ th category by the  $j$ th individual; and  $N$  = Number of constraints ranked by the  $j$ th individual.

The percentage position of each rank was converted into scores, referring to the table given by Garrett. For each constraint, the scores of individual respondents were added together and divided by the total number of respondents for whom scores were added. These mean scores for all the constraints were arranged in descending order and the most relevant constraints were identified.

## Results and Discussions

### ✓ The general profile of surveyed households

Based on the contribution of the annual livestock income to the total income, the surveyed households were classified into the livestock-based group and the non livestock-based group. Referring to the main characteristics of the surveyed households, the household heads in the non livestock-based group were older than those in the livestock-based group. They also had lower levels of education than those in the livestock-based group. Generally, the older farmers have lower levels of education than the younger ones. In addition, the farmers with the low levels of education likely perceive more limited access to the economic and social information than the others with the high levels of education. Both groups owned a small crop land area due to the high density population in Hai Duong. Most of the surveyed households simultaneously engaged in production of chicken, fattening pig and piglet. Selecting the diversification in livestock production, the farmers expected to reduce their risks. The number of livestock heads and the fish pond area of the livestock-based group were considerably higher than those of the non livestock-based group. Compared to the commercial farms, the livestock herd sizes of the surveyed households were considerably small because of their limited financial capital and land.

Table 1. Characteristics of surveyed households

Indicator	Livestock-based group (n=58)		Non livestock-based group (n=87)		P-value
	Mean	SD	Mean	SD	
Age of household head (Years old)	45	7.5	46	9.3	0.79
Education of household head (School years)	7.7	1.3	7.0	1.1	0.55
Area of crop land (1000 m <sup>2</sup> )	2.2	0.9	2.6	1.1	0.06
Area of fish pond (1000 m <sup>2</sup> )	3.5	2.4	1.4	1.2	0.000
Number of chicken (head)	223	76	183	70	0.005
Number of fattening pigs (head)	22.2	12.6	8.6	3.3	0.000
Number of piglets (head)	21.0	5.3	16.5	6.4	0.000

Source: Household survey, 2011.

### ✓ Cost and return analysis of livestock production

Regarding fattening, the volume of live pig of the livestock-based group and the non livestock-based group were 2404 and 805 kilos, respectively. The production cost of the livestock-based group was relatively higher than that of the non livestock-based group. The total production cost of the livestock-based group per a ton of live pig was 3527 Vietnamese Dong (VND), of which the variable cost occupied 97.6 %. Similarly, the non livestock-based group mainly invested variable inputs in pig production (98.5%). Of the total variable cost, the feed cost accounted for a main part, which was about 79 % for both groups.

Table 2. Production cost and return per 1 ton of live pig (Unit: 1000 VND)<sup>1</sup>

	Livestock-based group		Non livestock-based group		P-value
	Mean	SD	Mean	SD	
Variable cost	3527	318	3115	139	0.000
Fixed cost	84	48	47	36	0.000
Total cost	3611	330	3162	152	0.000
Gross output	4431	336	3886	280	0.000
Return to family labor	820	268	724	278	0.081

Source: Household survey, 2011.

Note: <sup>1</sup> 1000 VND was equivalent to 0.05 USD in the year 2011; <sup>2</sup>The variable cost excluded the family labor cost.

The fixed cost included the interest payment and depreciation. The livestock-based group borrowed the higher amount of money for feed purchasing and had the higher investments in pig shelter than the non livestock-based group did. Therefore, the fixed cost of the livestock-based group was higher than that of the non livestock-based group. On the other hand, the gross output of the livestock-based group was statistically higher than that of the non livestock-based group. The livestock-based group likely had a better knowledge of marketing than the non livestock-based group, which enabled the livestock-based group to sell their pig at a higher price. On average, the livestock-based group sold their pigs at 44.3 thousand VND per kilo whereas the non livestock-based group reached 38.8 thousand VND per kilo. Given a production unit, the livestock-based group generated a higher income than the non livestock-based group did, resulting from the higher input investment and the better experience in production and marketing of the livestock-based group.

Table 3. Production cost and return per 100 heads of chicken (Unit: 1000 VND)

	Livestock-based group		Non livestock-based group		P-value
	Mean	SD	Mean	SD	
Variable cost	8598	569	8498	521	0.286
Fixed cost	383	150	176	169	0.000
Total cost	8981	573	8674	568	0.002
Gross output	12325	1523	11040	1091	0.000
Return to family labor	3344	1504	2366	1063	0.000

Source: Household survey, 2011.

Like the pig production, the variable cost of chicken production occupied a dominant part of the total cost. The feed cost was the major element of variable cost. The livestock-based group had a considerably higher input expenditure and obtained a relatively higher gross output than the non livestock-based did. As a result, they generated a significantly higher return to family labor than the non livestock-based group received. Although both groups were familiar with chicken production, the differences in chicken production cost and economic return existed between the two groups. The reasons could be explained similarly as the pig production. In addition, the chicken weight of the livestock-based group and the non livestock-based group were 469 and 359 kilos, in turn. The average selling price of chicken was 59 thousand VND per kilo for the livestock-based group and 56 thousand VND per kilo for the non livestock-based group.

#### ✓ Contribution of livestock production to the income of surveyed households

The income from livestock production of the livestock-based group was 32.7 million VND, accounting for 36.3 % of the total income. The fish production also created an important

income source of the livestock-based group, which accounted for 28 % in the total income. The crop production and non-farm activities played a less important role in income generation of the livestock-based group than livestock and fish production. The income from livestock production of the non livestock-based group was 10.5 million VND, contributing 19.6 % to the total income. The income from crop production (48%) and non-farm activities (30.7 %) accounted for dominant parts of the total income of the non-livestock group. However, this group earned low benefit from those activities. It should be highlighted that the farmers in the non livestock-based group are mainly unskilled workers and dominantly engage in unregistered employments, which are not expected to create a stable income. On average, the monthly income per capita of the livestock-based group and the non livestock-based group were 1.68 million VND and 1.02 million VND, respectively. Notably, 60 % of surveyed households had lower monthly income than the average monthly income per capita of Hai Duong province in the year 2010 (1.30 million VND). Therefore, the improvement and expansion of livestock production would increase income for both groups due to the limited opportunities of increasing income from other activities.

#### ✓ **Main constraints of production and marketing**

The farmers perceived some main constraints related to both production and marketing. The ranking results showed that the livestock disease, the limited credit access, and the high and rapid increase in feed price were three leading problems. Following, the high volatility of output price and the insufficiency of market information and weak bargaining power were considered as the fourth and fifth problems. The main constraints negatively affected livestock production income in the surveyed year. Furthermore, it will impede livestock production in the next years.

Table 4. Garrett's ranking of constraints

Constraints	Mean score	Ranking position
Livestock diseases	69.3	I
Limited credit access	48.1	II
High and rapid increase in feed price	44.6	III
High volatility of output price	19.3	IV
Insufficiency of market information and weak bargaining power	16.5	V

Source: Household survey, 2011.

It was found that the poor disease prevention of farmers and the weak capability of the veterinary system in terms of veterinary service, disease detection and surveillance were the main reasons for the livestock disease. Although many farmers had participated in the technical training class, their knowledge of disease prevention was still limited. The portion of farmers, who did not apply pig vaccination, was 15 % for the livestock-based group and 35 % for the non livestock-based group. In addition, the epidemic outspreading was worsened because some farmers tended to sell their sick or dead livestock to recover a part of their capital. Concerning the veterinary system, it had a network from the provincial and district level to communal levels. At the grass-root level, the private veterinary workers who worked as veterinary shopkeepers mainly provided veterinary services to farmers. However, most of them had low training levels. In addition, they commonly provided vaccines kept in the poor condition to farmers. The veterinary system was mainly a passive surveillance, reacting to disease problems. Notably, the smuggled chickens from China were also a serious problem leading to occurrence and the outbreak of disease. The bad management of the veterinary system partly caused the existence of the smuggling of chickens.

Regarding credit access, the formal sector, which mainly provided credit for the agricultural production, did not meet the credit needs of livestock producers. Vietnam Bank for Agriculture and Rural Development (VBARD) and People Credit Funds (PCFs), two

commercial banks, basically supplied credit on the requirement of physical collateral. Despite having credit need for feed purchasing, 47.6 % of surveyed households did not apply for borrowing money from commercial banks due to being afraid of being refused, being afraid of risk, lack of physical collateral and high interest rate. Of the surveyed households, for 13.8 % was approved the full required amount of loans, for 28.3 % was approved a part of required amount of loan and for 10.3 % was rejected the loan request. The credit policy was promulgated so that VBARD was responsible for supplying a loan of less than 10 million VND without the requirement of physical collateral. Practically, it did not operate efficiently. Many farmers still had a limited access to information on the credit programs. Consequently, many farmers without physical collaterals did obtain credit from neither VBARD nor PCFs. Furthermore, the stronger credit needs of farmers were more rationed by VBARD and PCFs. Due to a limited access to credit, the credit constrained households accounted for 71.7 % of surveyed households. It revealed that a large demand for credit of livestock producers exists.

On the other hand, the farmers had gradually replaced the traditional feed from crop with the industrial feed for their livestock. Thus, the industrial feed was mainly used for livestock production. The surveyed data from households showed that from January 2010 to December 2011 the pig and chicken industrial feed prices increased by 37.5% and 41 %, respectively. It was reported that Vietnam imported 90-95 % of dried soybean cakes and fish powder, 50% of corn, 80% of premixes and 100 % of minerals and vitamins (SBOV, 2012). In the last few years, there has been a sharp increase in the price of many raw materials used in livestock feed production. The heavy dependence on imported raw material ingredients and high imported taxes has caused a high and rapid increasing in feed price. It would seem that the changes in costs of raw material inputs were passed to the livestock producers (Phuong et al., 2010).

About 81 % of the surveyed farmers reported that market price fluctuation was one of the main negative factors affecting their livestock production income. From the beginning of 2010 to the end of 2011, the chicken price and pig price varied from 50 to 71 thousand VND per kilo and 32 to 65 thousand VND per kilo, respectively. Notably, both chicken and pig prices had monthly volatility. The smuggling of chicken from China was one of the main reasons for sudden reduction in chicken price. In addition, in the first months of 2010, the price of live pig sharply went down due to the pig disease outbreak. After 2-3 months, the pig price gradually increased. While pig price did not show a strong pattern, the pig feed price increased continuously.

A lack of an organized livestock marketplace infrastructure means that farmers usually dealt with buyers on an individual basis (Alejandro et al., 2003). In the study site, the collectors living in or outside the villages of farmers generally provided market information to the farmers. The market information network was not organized systematically. Many surveyed farmers did not have many choices to sell their livestock at a fair price because of the insufficiency of market information. All surveyed farmers individually sold their livestock to collectors at the farm gate. 78 % of surveyed farmers reported that they were not satisfied with their selling price. The lack of cooperation among livestock producers in both production and marketing was detected as a dominant reason for a weak bargaining power of the individual farmer.

Some scenarios related to changes in pig feed price and pig price were taken to examine the influence of the increasing feed price and reducing pig price on the income of producers (Table 5). It was assumed that the feed price increased by 5 % and 10 % for the first and second scenarios, respectively. The return to family labor of the first and second scenarios consequently, decreased by 15.4 % and 30.8 %, in turn. The third and fourth assumptions

were that the price of pig reduced by 5% and 10%. As a result, the return to family labor of the third and fourth cases declined by 29.8% and 55.5 %, respectively. Obviously, the small increase in feed price or the small reduction in pig price caused a high reduction in economic return to family labor.

Table 5. A simulation with cost and return per 1 ton of live pig for the livestock-based group (Unit: 1000 VND)

Scenarios	Gross output	Feed cost	Return to family labor
<b>Based (average of 2010)</b>	<b>4429</b>	<b>2674</b>	<b>866</b>
Increase in feed price by 5%	4429	2807	732
Increase in feed price by 10%	4429	2941	599
Reduction in pig price by 5%	4204	2674	607
Reduction in pig price by 10%	3986	2674	385

Source: Household survey, 2011.

### Conclusions

Empirically, the study showed that livestock production contributed significant parts to the household income for both the livestock-based group and the non livestock-based group. The high investment of inputs and the considerable experience in production and marketing created a higher income for the livestock-based group than that of the non livestock-based group. Five main constraints of production and marketing, perceived by farmers, were respectively ranked as the livestock disease, the limited credit access, the high and rapid increase in feed price, the high volatility of output price, and the insufficiency of market information and weak bargaining power. To enhance livestock production and marketing by farmers, the disease prevention and the veterinary system should be improved to avoid a passive surveillance and to reduce risk related to livestock production. In addition, the information on credit programs should be provided to farmers efficiently. The credit supply from the formal sector needs to increase for a better credit access of livestock producers. Besides, the government's role in facilitating the domestic supply of feed raw materials should be strengthened to create a stable feed price. Moreover, the livestock producers should work together in groups to overcome both production and marketing constraints.

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## PERSPECTIVES OF RURAL AREAS IN SERBIA

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### Abstract

There are one million of unemployed persons in Serbia nowadays. At the same time, every fourth village, of 4,600 villages or 1,200 of them in Serbia is on the way to disappear. One and a half decade from now, only monuments are to stay, as a testimony of their recent existence. And while people are without jobs and villages are disappearing, opportunities for reducing negative trends in Serbian villages and development in rural areas are not exploited. More than half workers, who lost their jobs, could be employed in rural areas in Serbia. And, not only the workers, who lost their jobs due to structural unsuitableness of production, years of sanctions and transition crisis, but the workers who lost their jobs in companies in which they worked before NATO bombardment. All these results lie in the fact that workers are still with one foot in the peasant footwear *Opanak* and the other in a modern shoe. However, the return to rural areas of the country doesn't imply returning to hoes and tractors, but employing workers for agricultural and similar jobs in: forestry, water management, service activities, handcrafting, household industry, infrastructural, small and medium enterprises (there are 220,000 of SMEs, and the aim is 400,000), not harming ecological counterbalance.

**Keywords:** village, soil, deagrarianization, lack of perspectives

### The fastest deagrarianization in the world

Yugoslavia had the fastest deagrarianization in the world. For the last 50 years of the XX century eight million people have moved from rural areas to cities. The process like this lasted near 150 years in the world. The exodus was continued in the last decade of the XX century when the war broke out and persuaded near 400,000 from Croatia, Bosnia and Herzegovina and Kosovo and Metohia to move to Serbia. Upon the WWII there had happened huge changes in the rural areas. These changes are an integrated part of the universal global process of modernization of a society, its industrialization and urbanization. These changes also implied the ruling ideological paradigm of the then system being erected on that paradigm on the territory of the former Yugoslavia.

Today Serbia tries to find a new identity, a new paradigm of a social development, and this is the reason why we should not forget the rural areas and agriculture. As many times in history, even at the time being, the village and agriculture were two main pillars providing security and survival. Whenever it is difficult in Serbia people return to rural areas. The authorities consider that villages and recruiting people to do business there will get Serbia out of the crisis. However, it requires radically new relations of a society towards it, because there is nothing to do in towns, while in rural areas there is nobody to do that job!

Instead of being taken as the producers of cheap food, the agriculture and rural areas should be seen as an opportunity for the creation of demographic, natural, economic and socio-economic potentials. The main feature of a village today is a negative demographic trend. The birth rate is rapidly falling down. Therefore, this picture is the result of depopulation and social devastation of a numerous of areas in Serbia. The huge changes have appeared in the economic life of a Serbian village, which are not sufficiently examined and explained

although they have big social and economic consequences. In XX century a household in rural areas was defined by an ideological category of a maximum, which turned out to be an irrational issue. There is no such maximum today, but there is a few people having their big households going up to several thousands of hectares. An average field in Serbia amounts to three hectare and it is an additional income to the people living in the rural areas. In XX century the industrialization and urbanization produced 'civilization revolution'. It changed the picture of the world with the people living in rural areas, their values and morality, culture and life aspirations.

### **Traditional way of living**

Up to the WWII Yugoslavia was an agrarian and traditional country. Over 80 percent of its population lived in rural areas. Their entire world was focused on their land, household and family. After the WWII, under the pressure of the global processes there was created a new vision of a society, there was industrial revolution bringing changes. The industrialized households increased the goods, became market-oriented, and changed its opinion on the future of agribusiness. They have far more capital, knowledge and strength in comparison to traditional households. The industrialization reduced manual job, then the number of uneducated people and the employed with the combines had the features of industrial workers. It resulted in the increase of the number of inhabitants in urban areas from 21 to 50 percent for the last half of a century!

The latest data reveal that the Republic of Serbia currently has 5,113,000 hectares of agricultural areas or 0.68 hectare per capita. Out of that 4,252,000 hectare is arable land or 0.57 hectare per capita. According to the census from 2012 the agricultural households currently cultivate 3,355,859 hectares. In Serbia there are 908,990 beef, 3.4 million pigs, 1.73 million sheep, 235,576 goats, 26.6 million poultry and there are 673,651 associations keeping bees. When it comes to the mechanization there are 408,734 tractors and near 25,000 combines. The mechanization in Serbia is near 25 years old. Up to the transition, near 85 percent of the property was privately-owned, whereas all areas in Serbia are privatized (it is taken that around 400,000 hectares are state-owned). According to the census of the agriculture in 2012 Serbia totally has 631,122 agricultural households. Out of that 628,555 are agricultural and 2,567 (or 0.42 percent) are the privately-owned households, while two million people in Serbia are dealing with food production.

### **An agrarian country**

Today Serbia is considered to be a poor agrarian country. The agriculture with following activities makes up near 40 percent of the GDP. It could be proved by data saying that one man produces food enough for 15 people, while in Germany even 152 persons, in France 77, Austria 56, Slovenia 25, whereas the average in the EU is between 50 and 80 inhabitants. The production of food in Serbia is far below the food production in the EU. In Serbia annual consumption of pork meat amounts to 15.4 kilograms (in the EU this figure is 32 kilograms). The consumption of beef in Serbia is 4.4 kilograms, in the EU 15 kilograms. In Serbia annual consumption of milk per capita is 56 liters, while in the EU it is 100 liters. The greatest difference is in the butter, the consumption in Serbia is 200 grams, and in the EU it is 4-5 kilograms! The share of food expenditure in an individual consumption in Serbia amounts even to 42.9 percent, which puts Serbia among the worst in the EU. It is the picture of poverty in general.

A family living in a village in Serbia is a kind of a social ruin, which its today's charm owns to the old social architecture. In the post war period the size of an agrarian family was shrunken, but it is still greater than a family living in a town. A family living in a rural area



today has two old persons and children. Lately, the number of members within a family has changed. The grown-up children separate from their parents and make their own families. The older people love where they are, while their children long for modernization. Thus the civilization has entered a village. The share of agrarian people in the total population of Yugoslavia has reduced from 73 percent in 1948 to 19 percent in 1981. On the other hand, the USA and Switzerland needed 90 years for this, France near 100 years, Japan 73, and Denmark 130 years. The data show that in Serbia the number of agrarian population amounts to 9.4 percent. Out of this number even 50 percent are people over 60 years, while only 11.5 percent are the young under the age of 20 (less than 62,000).

The economic-social institution 'rural development' is a relatively new developing theory. It is mostly connected with the countries of the so-called 'third world', at the time of big problems in relation to food-poverty-population. It brings us to a conclusion that the beginnings of rural development are connected with agribusiness. Whenever someone mentions rural development it is known that poor areas are in questions. It is the reason why an integral rural development as the youngest branch of the developing theory is treated as an engine of the economic and social progress. To find a more efficient solution to numerous problems appearing in these regions, it is needed the adequate proportion of three things: knowledge, technical and natural resources and capital.

#### **Returning to a village does not mean becoming a farmer**

Everything that was happening in this territory left big consequences on the Serbian village too. A Serbian village depicts a gloomy picture. For example, in Serbia there are 4,600 villages, and in a decade every fourth village or 1,200 of them will disappear from the map. The 86 percent of villages recorded the reduction of the number of inhabitants, and only 12 percent of them recorded a growth. In addition, in 986 villages have less than 100 people. In Serbia 2,000 villages do not have the post office, the road - as the connection with the world - is missing at the 500 villages, and some 400 of them do not have any store. In Serbia today there are over 200 villages without any person younger than 20 years, and more than a half of population lives in rural areas. In addition to this, in 230 villages there are no elementary schools, and in 2,760 of them there do not exist kindergarten, which indicates the greatest number of population living in villages throughout Serbia is missing a lot. Describing a Serbian village today, we should point out that two thirds of them do not have veterinary station although the agriculture is the main activity.

In Serbia around 40,000 people die more than they have been born every year. Serbia is in danger to become an agrarian country without villages and people living and working there. A Serbian village is specific for poverty of old people living there. These old people will die and villages in Serbia will remain empty. In Serbian villages the only lots getting enlarged are unfortunately cemeteries. Throughout Serbia a total of 40,000 houses are empty, and on 145,000 of them is written temporary empty. One of the main structural problems of Serbian society is the fast reduction of people living in rural areas (depopulation of rural areas) which exceeds the pace of decrease of agricultural population (deagrarianization). In 60 percent of rural areas has been reduced by moving and going for a better life.

Therefore, hard living conditions, the distance from urban areas, bad road network and low income are the main reasons for the reduction of the number of people living in rural areas. The rural areas in Serbia are neglected. The villages in Serbia do not serve for the food production only, but their inhabitants have to live a decent life. Therefore, a village needs to have an ambulance, a hairdresser, a pharmacy, a mechanic shop, a store and a cinema. Stagnation and demographic devastation of villages in Serbia could be stopped by the development policy of rural areas. Since the most of the people living in villages is dealing with agriculture (over 60 percent), the depopulation manifests itself as devastation of agriculture and all rural areas being too far from the towns and without industrial plants,

communal and social infrastructure and development perspectives. The population policy should be founded on relevant economic, agrarian, regional, developing and cultural policy, which is different from the existing one. In that sense, it is of great importance to improve unfavourable position of agriculture in relation to industry, to subject to decentralized model of industrialization and urbanization, to invest more in transport and communal infrastructure in rural areas, to ensure social insurance and to provide cultural education of agrarian people. This program would stop deagrarianization and depopulation.

#### **Old fellows and empty schools**

The surveys show that in Serbian villages there are near 260,000 bachelors and 100,000 spinsters. The reasons are numerous and consequences are catastrophic. It was embarrassing to be married to a farmer and to live in a village, so girls rather moved to towns and get married to doorkeepers working in factories and lived poorer. If every of these bachelors would have had a wife and a child, there would have been new 500,000 people, which is important for biological, demographic and economic reproduction of a village and society on the whole.

Too many people living in towns without job turned out to be bad situation at the time of transit. At that time many factories were closed and people were left in the streets. Out of ten workers being without job more than a half could work in rural areas of Serbia. It refers not only to those who are left without job due to the structural changes of production, years-long sanction and transit crisis, but to those being left without companies they were employed with before the NATO bombing in 1999. However, returning to rural areas does not mean returning to hoe and tractors, but it means dealing with agriculture, forestry, water management and various crafts activities. Namely, Serbia has 220,000 small and medium-sized enterprises. Every political party in its election campaign makes a promise that it will increase this number to 400,000 and open a million of new working places. These are only promises for now. The concept of an integral rural development, as a part of regional development policy, is antipode to ordinary industrialization and is based on overall development of rural areas where almost a half of population of Serbia lives in. The policy being founded on agribusiness and rural economy on the whole should be in function of the permanent orientation of the state and economic development policy being based on decentralization and balanced economic development. It is of particular importance today when Serbia has near million of the unemployed and restructuring of big companies leaving workers without job. The majority of them come from rural areas; some of them return to villages and try to start up a new life. In this way two problems should be solved: Serbian villages would be recovered, and people would be employed. The state should support this new development concept and finance it in the beginning, if necessary, and would take care of the balanced development of the country and the creation of equal conditions for all stakeholders in the market game. The concept of rural economy, as a part of the regional development policy, should directly involve the joint and non-agricultural households having arable land. It should be pointed out that the production at the households in rural areas is not market-oriented.

#### **European experiences**

In the last 25 years the European Union is increasingly getting to be focused on its rural areas and the policy of rural development. At the Second Conference that took place in Salzburg in 2003 it was brought conclusion that the assets from the European Agrarian Fund for the support of the rural development will be higher. The goal is to support the financing policy, rural and sustainable development.

In addition that the rural areas provide markets with agricultural and food products, they also could be good places for relaxation, tourism and healthy life. These advantages of rural areas are becoming more and more visible and significant. The EU Commission recognizes the need for new investments in the creation of rural development. Besides the primary agricultural production whose importance in the rural economy is increasingly falling down, there are new opportunities. Perhaps, people will start moving to rural areas in the future.

The EU projections say that there are many possibilities in rural areas: to organize varied types of production, to reside permanently and to enjoy in the nature and landscape. These are the main trends of the rural development of Europe in the years to come. The EU member states being less developed and the countries tending to join the EU are not able to carry out the activities in every rural area and to solve all possible problems there.

In Serbia there are near 2,000 different manifestations every year. Serbia does not have a sea, but its chance lies in the development of agriculture and rural areas. If a household would have had double-bed room and rent it to foreign guests for 20 euro 200 days in a year, it would have made up the revenues of 16,000 euro annually. If only 10 percent of the population in Serbia would have dealt with tourism, Serbia would have had the income of 1.6 billion euro annually, (the income from tourism in 2012 was one billion euro). The agro-economic and rural-social researches and projections of the international institutions show that the European agriculture in the years to come expects: the changes in the size and number of farms, the reforms in households and land quality, the higher utilization of biological, information technology, equal regional development, the raise of the food quality and standards, the meeting of the requirements of consumers, commercialization and vertical integration of farms, various public investments and greater support to agriculture and development of rural areas.

If every country wants to increase its national income, it needs macroeconomic and political stability, the increase of productivity through new technology, growth of real income in non-agricultural sector and more stimulation to the production. In that sense, there is a question whether the agriculture could be an engine of rural development, could smaller farms survive and could the rural non-agricultural economy contribute to the development of rural areas. In order to prevent any negative trend, Serbia should equalize the number of people living in rural areas, improve the transport and telecommunication infrastructure, raise the health care system, culture and education level, enable the employment in other sectors, increase the income resources from non-agricultural sectors and organize small farms in agricultural cooperatives. According to it there are five principles of rural development. They say that possibilities of rural development should be recognized, then the reduction of poverty should be carried out, the decentralization process should be accelerated, the role and responsibility of local self-government should be strengthened and the productive sector in rural development that will contribute to the growth of economy and the reduction of poverty should be built.

### **Conclusion**

The conclusion is that Serbian villages are at the point of surviving and disappearing. The devastating process of rural areas is too intensified and it appears in many different forms. In order to stop all negative trends, it should be created a new policy of rural development. Farms are not only areas for production, but the places for relaxation and enjoyment. The European experiences should serve us as examples in the creation of our policy of rural development that have to be adjusted to local resources and initiatives.

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**BIO-ECONOMIC ASSESSMENT OF BARLEY CHAIN: RELEVANCE AND SMART, SUSTAINABLE, INCLUSIVE TRIGGER EFFECTS IN THE SHORT CHAIN OF FOOD AND NON-FOOD PRODUCTS - A PROJECT 'S EXPLANATION**

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**Abstract**

In the affirmation of the Sustainable Development Strategy, the agricultural production has a crucial role in the success of the bio-economy (Conway and Barbier, 2013).

The European Commission adopted a strategic action plan called: “*Innovate for sustainable growth: a Bio-economy for Europe*” (OECD, 2011). The bio-economy will be one of the basic topics of the Horizon 2020 program. In this sense, very promising results are coming especially for the cycle of barley. Barley is a cultivation with large diffusion all around the world and with high capability of adaptation regarding to climate and soil variation (Asveld *et al.*, 2011).

Last year a University Network of Central Italy built a Research Project of National Interest entitled “Process and product innovation in the barley food chain for the improvement of quality and environmental sustainability of food and beverages”.

The main objective of the Project is to arrange a bioeconomic evaluation of the barley chain with a particular focus on proposals for the supply chain and to boost the role of barley for the initiation of bio-economic development processes at national and European level.

In this sense, the paper represents a first contribution about a specific part of the research project and, moreover, aims to deliver an example at the academic level about the innovative opportunities of bioeconomic research field.

**Keywords:** Bio-economy, Barley Production Cycle, Green Economy, Sustainable Development, Innovation

**Introduction**

The theory of Economics Welfares of Pigou in the permeate management system and the hierarchy of wealth distribution has put in crisis the development model of the I° Industrial Revolution by the first in-depth concrete analysis (Georghescu, 1971). The issue of environmental goods, (their increasing scarcity due to the gradual erosion of a quantitative model of development, market failure due to the presence of free riders in the use of public goods) was placed first in his large economic magnitude (Plihon *et al.*, 2009).

The review of the quantitative model of development, a quality led to the affirmation of the Strategy of Sustainable Development, have been evolved since the late 1970, in which the bio-economy and green economy are the most relevant options (Leonardi, 2010). The same, it's a pillar of topics, confirmed during the World Summit on Sustainable Development on

22-24<sup>th</sup> June 2012 in Rio de Janeiro. Agricultural production has a crucial role in the success of the bio-economy (Kitchen and Marsden, 2011). The relevance of this approach, to prospects of economic and social development in Europe, are now a new strategic pillar. Just on February 13<sup>th</sup> the European Commission adopted a strategy to move the European economy towards a greater and more sustainable use of renewable resources (European Commission Communication, 2011).

The Commission's strategy and action plan, "Innovate for sustainable growth: a Bio-economy for Europe", outlines a coherent, inter-sectorial and interdisciplinary approach to the problem. The goal is a more innovative and low emissions, reconciling the demands for sustainable agriculture and fisheries, food security and sustainable use of renewable biological resources for industrial purposes, while ensuring the protection of biodiversity and environment. The plan therefore focuses on three key areas: development of new technologies and processes for the bio-economy, the markets in developing and competitiveness in the fields of bio-economy (Fumagalli, 2007).

The bio-economy will be one of the basic topics for the Horizon 2020 program, and featured research and theoretical insights fascinating especially from 2000 onwards (Jordan *et al.*, 2007). The University of Perugia, with other universities of Central Italy, have created a national project to improve the barley chain and its utilizations and prospective. The main targets of the Research Project are the following:

- to continue, develop and complete the topics covered in the PRIN 2008 project "Barley and beer: product and process innovation";
- to evaluate and classify the raw materials features for cereal-based food, including both solid food (biscuits, pasta and bread) and beverages, in particular beer;
- to study the agricultural conditions for the production of foods with a high functional value through tests on open fields concerning the effect of organic and conventional low input cropping systems, the effect of nitrogen fertilization, and the effect of genotype;
- to arrange a bioeconomic evaluation of the barley chain with a particular focus on proposals for the supply chain. For this aspect the aim of the Project is to boost the role of barley for the initiation of bio-economic development processes at national and European level;

A list of specific objectives for each of the four units is following:

- Operative Unit 1- University of Perugia, Economic and Food Sciences Department (DSEEA)- Enhancement of Bakery products made through the use of high quality and sustainability barley. This Unit is divided in three Work-Packages: study of formulation of bakery products using flour high nutritional and functional value; study of agricultural conditions for the production of foods with a high function value; bioeconomic evaluation of the barley chain with a particular focus on strategies for the supply chain.
- Operative Unit 2 - University of Molise, Department of Food Science, Technology, Environment and Microbiology (UNIMOL) – Development of barley based products with high dietetic and nutritional value.
- Operative Unit 3 - University of Tuscia - Department for Innovation in Biological, Agri-Food and Forestry Systems (UNIVT) – Lager beer production: assessment of a novel enzyme- and membrane-based clarification process and lifecycle greenhouse gas emissions.
- Operative Unit 4 - University of Perugia, Italian Brewing Research Centre (CERB) - Improvement of barley supply chain through the use of organic raw materials process innovations and enhancement of by-products in the production of fermented beverages.

The approach is holistic and interdisciplinary and consequently the researcher group dispose of specific and complementary skills. The skills and/or pilot and pre-industrial plants, available from national and international structures will be used. The Research Unit are four. The authors are member of the Research Unit of Perugia University and their contribution is related to the bioeconomic evaluation of barley chain, with particular emphasis on strategies for the supply chain. The activity is divided in three phases. At the moment the activity is at the level of the kick-off but the expected results are surely attractive. The research units are divided in a Research Body-RB.

### **Materials and Methods**

Concerning the materials and methods the authors describing the activity of the Unit 1 –WP3 that is to them assigned. The research aims to boost the role of barley for the initiation of bio-economic development processes at national and European levels. This is a crop that is well suited to European and throughout the area for its characteristics of hardiness has a comparatively low environmental impact and the production (green or dry) has already shown considerable interest and potential uses ranging from seedling, grass juice, bread, cakes, packaging, bio-mass, till in the art of decorative design. The approach to the Tradi-Ovation (tradition-innovation) can stand for “Territory, Rural Areas, through Development, Innovation, Organization, valorization, user friendly, Technology, ICT sharing, Online Networking”.

Concerning the materials the units research intends to work closely with the technological options for process and product innovation that will emerge from the whole project. It aims to achieve a contribution to the overall results of the project so that those most technologically promising have been given the appropriate assessment to identify the character “of current execution” for a quick transferability and repeatability in the territories in which barley culture is already present and/or adoptable as new areas potentially suitable. The work is identified in three phases:

*Phase 1-* survey phase, with indexing of technical and economic contents of the various technological options and features of short chain and innovative packaging that will be activated in the project (3 months);

*Phase 2-* development and evaluation of bio-economic models at farm level and regional commercial and technological options adopted (3 months);

Phase 3- evaluation of the value chain at the supply and territorial chain (6 months).

In details:

Phase 1 - The first phase will take place, on the basis of Access sheets. All the technical and economic base in order to get in quick times to bio assessments (economic, financial, performance, recovery period, energy balance, impacts, sustainability) of the various technological options and features that the project proposes. These data will be important because the type and extent of the same will be derived is the operational models for assessing the next step.

*Phase 2* - For each product and merchant choice will be adopted an evaluation at the micro level by focusing on the following aspects: Economic balance; Balance sheet; Cost-benefit analysis and determination of IIR and NPV; Determination of the payback period; Determining, using the technique of funds and flows of energy balance of the process; Evaluation of environmental impacts, employment, export, import; Assessment of

sustainability according to some indexes of the economic, social, environmental, managerial and cultural consistency.

*Phase3* - The third phase will pass on the value chain analysis of each process seeing both at micro and at the macro territorial level. It will go to check in particular the ability and speed of liquidity creation process but with attention to the whole supply chain.

### **Results and Discussion**

This paper aim to explain an “exercise” transformed in project to create a model that can be replicated in other contexts. The research try to verify if in the chain of lifecycle of the barley production the capability level to stimulate the concrete best practice of the paradigm of sustainable development strategy, the affirmation of the green economy and the improvement of the imprinting of the agriculture to be a pillar of the new horizon of the bioeconomy.

The aim of the all project is to improve the innovation of products and process along all the lifecycle of barley. In this approach is possible to improve the traditional transformed products, but to introduce the experimentation for the innovative food and not food products. At the moment many countries, companies, cities and citizens recognize that this narrow concept of wealth is not sufficient to explain the extraordinary losses that are occurring, basing their assumptions about levels flourishing of science and more sophisticated economic analysis.

In the global approach the aim of the project is the study of raw materials with high nutritional value from barley chain used in the formulation of food and fermented beverages with improved sensory and functional properties to the satisfaction of consumers.

Furthermore, a specific result is the improvement of the management of food safety in the supply chain. Finally, the by-products obtained along the chain could assure greater environmental sustainability. Another target it's to enhance the barley grain for food use by process and product innovations and by the improvement of dietary and nutritional features, quality and safety of the products obtained (functional products with health claims in accordance with directives and validation of the European Food Safety Authority, EFSA).

The open field project activities intend to evaluate the effect on the environment, food safety and barley technological quality of different cultivation systems of barley for human consumption and therefore cover both the biscuits with high functional value production chain and the fermented beverages. The open field experimental activities aim to evaluate the effect of agronomic variables of environmental sustainability of cultivation, technological quality of grain used for the production of solid foods with a high functional value or fermented beverages and food safety.

Particular attention will be given to the effects of the agricultural phase on the grain's beta-glucans content; hopefully, the content of beta-glucans will be stressed to increase content for the production of biscuit, while it will be stressed to decrease the beta-glucans content when intended for the production of beverages.

The open field tests will provide the project with the grain produced with different farming processes and, above all, accurately plotted; this condition is essential to define correctly the impact of agricultural phase on the subsequent technological steps in the functional foods production.

The carbon foot-printing will be evaluated and measured on samples of: beer from different production scales (from industrial to hand-crafted) and different packaging (bottle, keg,



cans); dried pasta and biscuits on the basis of agronomic inputs. Finally, a specific result we expect by the study of chemical and nutritional features of spent grains that will be carried out, with particular attention to carbohydrates structure, antioxidant fraction, and their effects on performance, health and quality of growing lambs and rabbits meat.

### **Conclusion**

Over the past 25 years, while the world economy has more than doubled, up to 60 percent of the world's ecosystem services covered by the Millennium Ecosystem Assessment are being degraded or used unsustainably. Each year, 13 million hectares of the world's forests - the size of Greece - disappear. According to the UNEP Year Book of 2012, 24 percent of the total area has already suffered declining health and productivity in the quarter century due to unsustainable land use. Some types of conventional and intensive agriculture are causing soil erosion rates 100 times more than the rates at which the nature of the soil can be formed in the first place habitats such as forests, peat lands and grasslands in developing countries alone could be converted into arable land, losses aggravating the ecosystem life and biodiversity (Benner and Lofgren, 2007). Emissions of greenhouse gases continue to rise, pushing the planet towards the 2° C threshold beyond which the scientist some fear could become irreversible environmental changes.

Hence the need to think to root an economy that points to a new vision of wealth that does not mean regression return to the center but above all the man, nature and innovative services and efficient for a democracy more possibly happy (Meadows *et al.*, 1972). Flexible and inclusive economy, which provides a better quality of life for all within the ecological limits of the planet. Below we highlight the key principles on the Green Economy emerged from the work of various institutions and scholars (Carlson, 2007).

The European bioeconomy today has an annual turnover of about €2 trillion and employs more than 22 million people, approximately 9% of the total EU workforce. Its further development will significantly contribute to reducing the dependency on fossil resources and offers opportunities for growth and jobs (Morin, 2006). Building the bioeconomy is one of the great research and innovation challenges within Horizon 2020, the proposed European Union Framework Programme for Research and Innovation for the years 2014 - 2020.

The Bioeconomy Strategy “Innovating for Sustainable Growth: A Bioeconomy for Europe” adopted by the European Commission on 13 February 2012 wants to make this vision a reality for Europe by addressing bioeconomy related challenges in a comprehensive and sustainable manner (Tyndall *et al.*, 2011). Its implementation will build momentum for strengthening European competitiveness, making the bioeconomy a powerhouse for economic growth and job creation in Europe and beyond. The concept of the bioeconomy should be more strongly integrated into European policies. In particularly the Common Agricultural Policy should take the bioeconomy much more into account. This economic concept is composed of numerous new value chains to which farmers, fishermen, and forest and aquaculture managers will add significant value (UN-WECD, 1987).

This requires a higher degree of training for new skills and competences which undoubtedly will lead to higher earnings and the creation of new businesses. New facilities and infrastructure will be required to effectively use the available biomass resources. Investments in establishing and optimizing infrastructures and logistical capabilities are crucial to ensure that all biomass can be mobile. In particularly the target of the WP3, assigned at the authors, is to verify also how are the possible new products alongside all of the barley productive chain in the approach of low input process and reduce-reuse-recycle (3R Regulation).

This not for following the hypothesis of the degrowing economy but for leaving a true impulse for the creation of new high level of wealth by the agriculture in a sustainable way. In this option could be possible that the future generations, that in 2050 will be a 9 billion in our planet, are able to live in a desirable happiness era.

Particularly the WP3 of the Unit-1 of the Research Project has a revolutionary content and wants to be a step to contribute to transform the logic overtake approach of the measurement of the welfare of the Nations with the GNP (Gross National Product) in that more attractive of the GNH (Gross National Happiness) and to achieve a concrete target of the affirmation of the bioeconomy strategy.

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## IMPACT OF SOCIAL PROTECTION PROGRAMS ON ECONOMIC RESILIENCE OF POOR HOUSEHOLDS IN EASTERN PROVINCE OF RWANDA

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### Abstract

This paper analyses the contribution of three programs of poverty reduction in Rwanda. It uses data from different reports and surveys with beneficiary poor households of social protection programs. The activities developed by these programs have allowed the poor in general and the widows of genocide in the Eastern Province of Rwanda in particular to improve their socioeconomic conditions, notably access to education, accommodation and medical care. Moreover, the beneficiaries have developed activities that diversify households' revenues and improve their economic resilience.

**Keywords:** Social protection program, economic resilience, poor households, Rwanda

### Introduction

Rwanda is one of the poorest countries in world; it is classified 166<sup>th</sup> among 186 (Khalid, 2013). Rwanda is also one of the smallest countries in the world with 26.338 km<sup>2</sup>, and 10.8 millions of population. With an annual demographic growth rate estimated at 2.9 %, the Rwandan population would reach 15 million by 2020 and 20 million by 2030 (INSR, 2010). The poverty rate is 44.9 % and 24.1 % for extremely poverty (NISR, 2011)<sup>25</sup>. Agricultural sector is the pillar of the Rwandan economy with more than 80% of population engaged in agriculture. To Andy McKay (2007), 90 % of households own agricultural land and more than 60 % of them has less than 0.7 hectare. Rwanda's vision 2020 is a long term strategy of development that has been implemented since 2000. It is a crossroads that moves from the humanitarian assistance phase associated with the 1994 genocide into one sustainable development (Minecofin, 2000). Different social protection<sup>26</sup> programs have been set for poverty reduction. These include (1) the Public Fund for Genocide Survivors (FARG) that essentially uses 5% of total country budget. FARG supports education, health to orphans and direct support in cash, accommodation and financing of generating micro-project (GMP) for vulnerable households. FARG is a special social protection program for vulnerable Genocide Survivors; (2) the vision 2020 Umurenge Programme (VUP)—an integrated local development program which has three components: (i) Direct Support (DS) in cash for the poorest people who are unable to work, (ii) Public Works (PW)—this component promotes rural employment related to productive community assets<sup>27</sup>, development projects and (iii) Financial Services

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<sup>25</sup>Poverty threshold and extreme poverty are determined on the basis of 194 and 137 USD, respectively. The national Bank rate of exchange on July 17, 2013 was 1 USD = 650 966 Rwf.

<sup>26</sup> This paper uses the definition of social protection from the European report on development 2010: “A specific set of actions to address the vulnerability of people's life *through social insurance*, offering protection against risk and adversity throughout life; *through social assistance*, offering payments and transfers in kind to support and enable the poor; and *through inclusion efforts* that enhance the capability of the marginalized to access social insurance and assistance.”

<sup>27</sup> The community assets refer to road, sanitation and education infrastructures.

(FS) – which increase the access for the poor to financial services by providing loan at low interest rate; (3) Girinka Program known also as “one cow per one poor family”. The program enables poor households to own and manage an improved dairy cow that would help the family to better their livelihood through increased milk and meat production and increase of soil fertility for their crops, using available manure.

### **Materials and methods**

In this paper, the data have been collected by surveys of beneficiaries of the social programs protection on the one hand and on the other via interviews with local authorities and the managers of AVEGA. This concerned 18 genocide widows in two sectors of Rwamagana District in Eastern province of Rwanda which are in AVEGA<sup>28</sup> Association. These data have facilitated the analysis of socio-economic resilience at household level by comparing the widows’ socio-economic conditions before and after the launching of these programs. The other information comes from different reports. Secondary data are from the report of social protection programs at national level.

### **Results and discussion**

The outcomes are presented in first place compared to protection programs before the consolidation by survey data.

- a) The expenses of FARG allocated to vulnerable survivors’ problems since its inception in 1998 are evaluated at 124.879.174.000 Rwf; that’s 191.836.707, 29 USD (Primature, 2012). They concern all the interventions at national level and are presented as follows:

Figure 9: Expenses allocated to survivors problems,

C&IGP: Cash and income generating project

On its own, education uses 62. 2 % of FARG’s funds. It is a short or long term strategy of poverty reduction for households, as well as the whole nation. In fact, for the concerned period, on average for each year, 40428 and 2098 students have received FARG scholarship respectively in secondary schools and in university. However, it should be mentioned that scholarships are offered to children who excel in school, which limits the chance of those who do not get the required marks. The handicapped people or those in advanced age get a financial direct support for their survival. For the period between 1988 and 2012, the average of 24460 have received annual averages of 572459000 Rwf a year. Regarding accommodation, 727 houses, of an average value of 1 598914 Rwf in 2012.

The widows we interviewed appreciated FARG support, mostly in the domain of education. They expected a bright future hoping their children will help them once they will have completed their studies.

- b) In contrast with FARG that specifically focuses on the problems of Genocide Survivors, VUP- 2020 deals with all poor households’ social protection. Its achievements are summarized below:

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<sup>28</sup> AVEGA: Association of widows survivors of genocide -Agahozo

Table 1 : Support to the poor in the form of social protection, in (000)

Components	2009-2010	2010-2011	2011-2012	Total	%
Direct support	1,396,360	2,800,564	4,035,706	8,232,630	20.32
Financial services	4,324,079	4,132,532	3,851,633	12,308,244	30.38
Public works	6,759,195	8,977,224	4,233,478	19,969,897	49.30
Total	12,479,634	15,910,320	12,120,817	40,510,771	100

Source : Reports RLDSF<sup>29</sup> (2009-2012)

The above table highlights that 40.5 billion have been directly injected in poor households via VUP-2020 program. Finance comes from the Rwandan government (54 %) and financial sponsors (46 %)<sup>30</sup>.

Direct support covers 20.32 % of the fund, i.e financial transfers to households identified as poor. For the period between 2011–2012, 27631 households of 62854 members got direct support. Among them, those managed by women are 18.660 against 8.971 ruled by men. The financial services represent 30.38 % of the total amount. The beneficiaries of the credits for the period of 2011–2012 are evaluated at 14 956, with 8 459 personal credits, 6 394 offered to groups of people and 103 to cooperatives. Women represent 48 % of the total. Credits offered by VUP program during 2010 and 2011 represent respectively 13 % and 11 % (BNR, 2011)<sup>31</sup>. Remunerations from public works have covered 49.30 % of the total amount. The jobs created for the same period are 94 397 of which 46 % for women. The average remuneration for these jobs was 44 847 Rwf.

Do achievements reduce poverty in beneficiaries' households? What are the other social economic effects induced by the activities of Umurenge 2020-VUP programme?

Regarding the data of the integral survey on life conditions of households (EICV1, 2000 and EICV2, 2005) and the outcomes of other studies notably those of the EU carried out in 2009 for measuring the impact of VUP-2020 programme, it becomes clear that the average income of the poor is estimated at 146 Frw/day/adult in 2000 and 150 Rwf/day/adult in 2005/2006 (EICV, 1 and 2). With the new VUP components, a household beneficiary of direct transfer receives amounts calculated as follows: 250 Rwf for the household head, 150 Rwf for the second head in hierarchy (the wife for instance) and 100 Rwf for other members of the household (maximum 3). The household of 5 persons and above gets 700 Rwf a day (21 000 Rwf /monthly and 252 000 Rwf /annually). This amount supplements the revenue the poor used to earn from their own economic activities.

The VUP components allow the development of exchanges in rural areas under the effects of trainings. Indeed, the data collected from EICV3 (2011) confirm that 8% of households at national level believe to have increased their monetary income from community works activities and the credits offered by VUP 2020 programme. Only 1% declares their revenues vary because of the component « direct support ». This is well justified as the latter concerns the poorest households, the ones without even a possibility in agriculture (household without arable land). Moreover, as these are the people with no working skills, the direct support is directly used for basic needs (food, clothes,...). The results of the assessment done by the EU in 2009 on 120 collective projects and 60 individual ones of households in the context of the UE-VUP project, the direct or indirect beneficiaries of Ubudehe project are evaluated at 1,4

<sup>29</sup> RLDSF : Rwanda Local Development Support Fund

<sup>30</sup> Data collected from the report of RLDSF (2012).

<sup>31</sup> For this comparison, the credits portfolio of the IMF in Rwanda in 2010 and 2011 represent respectively 32,3 and 37,8 billions of Rwf. The data are from the report of the National Bank of Rwanda for the year 2011.

millions, that's 20% of the Rwandan population. The results of the study also confirm that 96.6% of the interviewees have improved their food. However, 100 % of households say they are unable to pay school fees for their children and mostly in higher education. Globally, the income has improved (view of 95% of respondents) but remains poor. The impact of VUP actions is finally analyzed in comparison with its component « financial services». This helped to check if the credits offered to the poor are well reimbursed. The table below depicts the situation of 2009/2010.

Table 2 : Credits offered by VUP financial services (in 000)

Province	OC	TR	RC	% UC
Kigali	256,947	262,087	142,846	54.50
East	1,088,015	1,116,361	553,299	49.56
West	1,035,813	1,058,595	642,317	60.68
South	1,132,432	1,073,985	557,234	51.88
North	805,541	820,513	460,570	56.13
Total	4,318,748	4,331,541	2,356,266	54.40

Source : RLSDF (2009/2010)

OC : Offered Credit, TR: Total Required, RC : Reimbursed Credit, UC: Unpaid Credit

The outstanding loan at the end of the year is only reimbursed at 54.40 %. This is a big problem for institutions like banks, because their main objectives are profitability, liquidity and solvency. But for a development project such as the one supporting the poor (social protection), this is not very alarming as the objective is to educate the poor on better managing finances in terms of savings and investment and this takes time. Failures in reimbursement are attributed to the management system (projects study, recovery mode, deadline for reimbursement: a year maximum).

c) The program One Cow per One Poor Family (*Girinka munyarwanda*), like FARG and VUP-2020 program, is also one of social protection programs for poverty reduction in Rwanda. It reinforces the productive physical capital by owning a milky cow. Conversely to the two previous programs, Girinka does not target the poorest households. The choice of the beneficiaries considers the capacity to feed the cow in stalling, an obligatory practice since 2006 (EDPRS, 2008). All in all, in order to get a cow, one's obliged to own land comprised between 0.3 and 0.7 ha (RARDA, 2006) in order to produce fodder and have another part for food producing. There exist a tacit contract between the beneficiary and the local authority regarding the redistribution (*Kwitura*) of the first calf to another poor household of the village in order to perpetuate the process. For the period between 2006 and 2012, 114 803 cows have been given by Girinka, 25% of them to the Eastern Province (RADA, 2012).

The outcomes of the study conducted by Argent *et al.* (2013) show that the cows distributed to the poor through Girinka program have improved food by the consumption of milk, diversified revenues by selling milk. The cows have also helped to get farm fertilizers and the increase of productive assets thanks to new offspring. These authors have equally found that only 31% of the cows produced milk (whereas the expectations were 67 %). It is worth mentioning that performance production for the distributed cows is less compared to the expectations. In fact, it is evaluated at 31.1 % in comparison with the standard of 67 %. These differences are explained by the fact that Girinka has distributed many local cows of 'Ankole' race that produce less milk (almost 2 litres/day) but they easily adapt to the climate. Secondly, these exotic races (hybrid and pure) produce more milk, but lack sufficient fodder and water. Finally, 95 % of the beneficiaries of Girinka program use organic manure in their

fields and 20 % say they sell a part of their manure. Globally, 90 % of the beneficiaries ascertain that their socioeconomic conditions have been improved by detaining a cow.

- d) The results of a study conducted in 2012 with the widows' survivors of the genocide and beneficiaries of the programs of social protection are represented in the table below:

Figure 10 : Support to widows of social protection programs

This support has allowed the widows to develop their economic resilience, notably thanks to the diversification of income generating activities. These activities include agriculture, farming and small business. Only one widow practices business as income generating activity. Among all the respondents, she is the most economically resilient. She's a well known personality in the city of Nyagasambu. In general, agriculture remains the most important activity for the widows. It is practiced by more than 96.67% of the respondents, mainly subsistence agriculture involving polyculture. It is associated with farming (70% of respondents) and with small business (40%). Agriculture is the unique activity practiced by 10% of the widows. Regarding the cows distributed to the widows, they have improved the widows' food supply, helped the production of fertilizers and the increase of agricultural production. Finally, VUP credits allow the widows to develop commercial activities in the city of Nyagasambu (Eastern Province).

### Conclusion

The programs of social protection that have been discussed in this survey are important tools for poverty reduction in Rwanda. In fact, they allow the poor to have access to education, accommodation, medical care, job and the financing of small income generating projects and productive assets (mainly cows).

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## **INTRODUCTION TO THE DEVELOPMENT OF AN ON-LINE STUDY SUPPORT MATERIAL FOR AGRICULTURAL EDUCATION PROGRAM**

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### **Abstract**

This article deals with the teaching model of ESP (English for Specific Purposes) for students of the first year of Viticulture, Oenology and Pomology. The model is based on blended learning to assist students in successfully mastering the course. It is realized as a combination of a face-to-face environment and online learning in the Moodle Learning Management System (LMS) on the B2 level of the Common European Framework of References for Languages. An ESP coursebook has been designed in collaboration with specialist professors, based on a needs analysis with special emphasis on developing the skills needed for mastery in ESP.

Online study support material is focused on the development of agricultural terminology, reading and listening comprehension, grammar activities and work with up-to-date authentic audio-visual materials with the aim of developing students' language competences and support the professional growth of future viticulturists, oenologists and pomologists. Online support material can serve not only the students it was primarily intended for but also the students and academic staff of other polytechnics and universities with similar curriculum.

**Key words:** E-learning, English for Specific Purposes, Agriculture, Croatia

### **Introduction**

In recent years E-learning has been introduced as an established field to support or substitute traditional forms of teaching and learning. Language teachers have realized the huge potential of technology for providing linguistic and interactive content for their learners.

English for Specific Purposes (ESP) is a kind of English language teaching that is focused on developing specific skills of the learner. Dudley–Evans and St. John (1998) claimed that the ESP practitioner embraces multiple roles, such as teacher, course and material developer, collaborator, researcher and evaluator.

At present the situation of learning a foreign language at a tertiary level in Croatia is the continuation of language teaching from secondary school (students leaving school around B2 level of the Common European Framework of References for Languages) and starting tertiary English at this level.

The English language instruction program at the Agricultural Department of the University of Applied Sciences in Požega amounts to 60 hours, distributed in the first two semesters of studies. The language groups differ not only in size but also in ability.

Today's students are named as the New Learners, the Digital Native or the Next Generation which suggests a fundamental difference in the way they approach knowledge acquisition, problem solving, and moving into the workforce (Dobbins, 2005, Dziuban & Hartman, 2004). Having this in mind the teacher must be cautious in choosing content, materials and methods. This article will share local Croatian experience of developing and creating electronic teaching aids and assessment materials to improve the quality of study and self-study within the LMS Moodle software.

### **Background and Objective**

Teaching English for Specific Purposes involves the same set of skills as learning General English and it also focuses on acquisition of specialized lexicon and registers. All the students have to be familiar with specialized vocabulary and they have to be prepared for some common situations such as going for an interview or making presentations. In addition, they should be proficient enough to do research for their thesis which means that they should be able to find, read and evaluate various English language sources. Therefore, the teachers must be very careful in choosing content, materials and methods that would motivate students not only to participate in the lectures but also to work on their own. Modern online technologies are a great potential for teachers who have to adapt to new trends and incorporate them into their lesson plans.

Hutchinson and Waters (1989) suggested that teachers follow four basic precepts when they start developing their ESP materials. They are: suitability for the proficiency level, relevance to learners' needs, creativity in tasks/activities and discursive strategies, and stimulation of the target speech acts. Such materials do not teach, but facilitate the learning process; present a clear and coherent unit structure; are in consonance with pedagogical approaches; and offer problem-solving tasks, as well as lay down models for language use. Having in mind the four concepts we compiled the material which was used in a face-to-face environment. After that we started planning the course with online support material for closing the possible knowledge gap, testing and evaluation assessment that would enable the blended teaching approach which depends on the needs of students and the expected learning outcome. Hinkelman (2005) points out that the aim of blended learning is to span this continuum, define or describe tasks that encompass a multitude of venues: classroom, home, laboratory, and field.

The main objective of our blended learning model is creating complex online study support material for the English Course for Students of Viticulture, Oenology and Pomology. The course is designed to support an interactive form of teaching by means of multimedia application. The use of information and communication technologies will enable the students to fulfil specialist demands in English at the qualitatively higher level.

### **Design and development of material**

At present English Language for Students of Viticulture, Oenology and Pomology is taught in the form of contact teaching (once a week for 90 minutes) with the use of coursebook (developed in collaboration with specialist professors, based on a needs analysis with the focus on developing the skills needed for mastery in ESP), handouts and additional audio-visual materials. The large number of students in a group as well as the available time for lessons does not enable the use of authentic materials and additional grammar exercises which are presented in the online study support material which motivates students to improve their knowledge by doing extra work in weak areas, to do their homework and gives them an increased sense of autonomy. Computer technology enables them to complete additional tasks, check their progress and get their feedback. The course is focused on the development of agricultural terminology, on reading comprehension, listening comprehension and work with up-to-date authentic audio-visual materials. It is composed of fifteen module courses available in the Learning Management System (LMS) Moodle with the following topics:

Meeting New Needs of EU Farmers  
Genetic Engineering  
The History of Grapevine Cultivation  
Grapevine (Grape Varieties, The Annual Lifecycle of Grapevines)  
The Art of Winemaking (Turning Grapes into Wine, Fermentation, Aging, Barrels, Bottling, Cork)  
Wine Laws (Wine Law of the Republic of Croatia, Wine Labelling)  
Secrets of Senses (The Smell of Wine, The Taste of Wine, The Colour of Wine, Aroma Wheel, Wine and Health)  
Serving and Drinking Tips (Bringing Wine to the Correct Temperature, Decanting Makes a Difference, Content Determines the Shape, Wine Glasses)  
Fruit (How Botanists Classify Fruit)  
Instructions for Planting an Orchard  
Propagation (Vegetative Propagation, Whip Grafting, Cleft and Bark Grafting)  
Caring for the Crop (Beneficial Insects)  
Harvesting (Manual or Mechanical Harvesting, Winter Harvest of Grapes)  
Marketing Fruit  
Fruit Mosaic (The Apple, The Big Apple, The Plum, The Pear)

The content of the course is as follows:

Lead-in

Understanding vocabulary (exercises with key words and definitions)

Before you read (reading, connecting, making predictions, quiz)

Reading authentic material concerning the topic (a follow-up to the unit and work with the textbook)

Autocorrective exercises such as filling in the gaps, true/false, multiple choice, word formation, matching, synonyms/antonyms, homonyms/homophones etc.)

Listening comprehension exercises for recycling specialist vocabulary

Video transcript with corresponding exercises to understand spoken English better

On-line handing of written assignments (translations, letters, mind maps etc.) in some modules

Grammar activities (review and recycling of grammar corresponding to tasks from the textbook)

Self-testing exercises (exercises for training purposes with limited or unlimited number of possibilities or a credit test with just one try and time limit)

Final assessment

Read on (list of additional available resources)

Each module serves as a support to the coursebook lectures. Supporting the lecture means that some topics for repeating or deepening are optional, the others are an integrated part of the lecture and therefore, their use is mandatory.

Online study support material development is an on-going process with teachers who are constant learners and our experience, our views, teaching concepts and methodological knowledge is constantly changing in order to meet the needs of the new generations of students. Equally important is the course evaluation which is the last, but not the least important stage because it helps to improve and promote the effectiveness of the online study support material.

Figure 1: A page from Moodle English Course for Students of Viticulture, Oenology and Pomology showing a fill-in exercise intended for students' vocabulary practice

Figure 2: A page from Moodle English Course for Students of Viticulture, Oenology and Pomology showing a before you read brainstorming exercise

Figure 3: A page from Moodle English Course for Students of Viticulture, Oenology and Pomology showing a link with a video on the Internet with a listening comprehension exercise

Figure 3: A page from Moodle English Course for Students of Viticulture, Oenology and Pomology showing a grammar booster exercise to recycle articles

### **Conclusions**

On-line study support components for Students of Viticulture, Oenology and Pomology at the University of Applied Sciences in Požega, Croatia have been designed to complement the coursebook, deepen and extend contact with and among students. Teachers can distribute some of the course activities throughout the semester, with students accessing course material and completing certain tasks online. Various exercises with immediate feedback give students an insight into the areas they need to practice more. They can access a range of interactive activities, tasks and tests when they are online at home or in the computer lab whenever and wherever they have access to the Internet.

This paper has attempted to explain some issues for on-line study support material preparation, design, development and evaluation. It is an on-going process in which the teacher makes changes to meet the students' needs and interests and helps them to become more independent with flexible solutions in a learning environment.

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## THE RATIO OF REGIONAL AND RURAL DEVELOPMENT IN MONTENEGRO

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### Abstract

This paper suggests a selective basic problem and theoretical framework of regional development of Montenegro as well as development of rural areas. Land area of Montenegro is 13 812 square km with 4800 square km of inner sea. According to the latest census (2011) it has a population of 672 656 inhabitants. The average population density is 48 inhabitants per square km. Montenegro is administratively divided into 22 municipalities. Geographically it can be divided into three regions. In agriculture there are available resources of about 0.80 ha and 0.29 ha of agricultural and arable land per capita, which is significantly above the available resources in most European countries. Montenegro has significant tourism potential in rural areas, and therefore, rural tourism is a strategic priority. Based on in details analyzed attitudes toward handling and the importance of a correlation of regional and rural development, and also the need for planning the development process, integrative planning based on the principles of sustainable development is a conceptual starting point and the assumption of application program. Balanced spatial development does not mean that Montenegro should be regarded as a single territory with the same problems and opportunities, but we need to support spatial development focusing on regional specificities and thus mitigating the differences in development.

**Keywords:** region, development, ecology, rural

### The ratio of regional and rural development in Montenegro

#### Introduction

Montenegro covers the area between 41 °- 39 and 43 ° 33 north latitude and between 18 ° - 26 and 20 ° -21 east longitude. So, according to latitude, Montenegro is the southernmost part of Europe - the Mediterranean, one of the most beautiful parts of both Europe and the world. Distance between the southernmost and northernmost points of Montenegro 200 km as the crow flies, and between the westernmost and easternmost points 173 km. According to the latest census (2011) it has a population of 672 656 inhabitants. The geographical position of Montenegro is more important than the size of its territory or its population. Montenegro is situated on the crossroads of two major geographical units - Dinarides and the middle of the Mediterranean. Length of its coastline is 316 km and the surface of the inner sea is 4 800 km<sup>2</sup>.

Length of the Montenegrin coast is 316 km coastline, although including islands and the part of the river Bojana it is 339.2 km. The coefficient of coastal dispersion is 3.51. (Bakic at al, 2009). The planned level of development of transport system, whose primary role is to provide the conditions for balanced spatial development through better international, regional, inter-municipal and local networking – is not accomplished. Agriculture, along with tourism and services, is the top priority of economic development in Montenegro. In the total GDP, agriculture accounts for about 15%, while it constantly employs about 9% of the active workforce. Many households are farmers on small holdings (about 5 ha on average), so it is a primary or supplementary source of income for over 60,000 households.

This paper has applied and the theoretical character which looks at the planning, legal and institutional framework for the protection of Montenegro. It aims to present understanding of the geographic features of Montenegro. Application of regionalization resulted from analytical - systematic approach to the main features of the research. Thus, regionalism implies understanding of space from the angle of hydrology, geomorphology, tourism and urban planning, history and economy.

By choosing the method of regionalization, it is to comply with its basic principles: complexity and elementary typicality, and homogeneity (unity) throughout the territory. This is the only way to understand the observed territorial unit and investigate such unity. Administrative division is the actual spatial expression of the entire political and economic life, no matter whether it presents the best framework for his organization.

### **Material and Methods**

In the basic methodological apparatus regionalization method was used. The authors first used a method of geosystem analysis, and then statistical analysis. Evaluation methods were used to determine the significance of geological heritage for tourism, recreation, and culture. Systematization and classification method was used to arrive at the development of correlations among the forms in space. The authors used the method of correlation in order not to have to carry out research of potential. According to natural conditions, level of development, agriculture, rural development, tourism and recreation has a distinct regional differentiation. The need for balanced development with social and environmental requirements is a requirement for all regions. Coast (an area in which a small area of concentrated natural and created values) has become very attractive for tourists but also for the Montenegrins who migrate to the more economically prosperous and expansive areas. In the Central region specific segments of tourism such as cultural and religious have great potential. (Bakic at al, 2009)

### **Results and Discussion**

Montenegro has a varied relief, which consists of high mountain ranges (Orjen, Rumija, Bjelasica, Komovi, Durmitor, Prokletije etc.), valleys (Zetska, Grbaljska, Belopavlicka ...) intersected by a number of rivers that make canyons (Moraca, Lim, Tara, Cehotina, ...). Such an arrangement of relief and the influence of climatic factors is extremely unfavorable for the construction and maintenance of infrastructure in particular. If we add to this the fact that over 55% of the total surface of Montenegro is, - 1000 m above sea level,- and about 24% of Montenegro is situated on a slope greater than 30%, we get an extremely complex and difficult framework for building and maintenance of transport infrastructure. Administratively Montenegro is divided into 22 municipalities. Geographically Montenegro can be divided into three regions: coastal region, central region and northern region.

1 Coastal area, includes the coastal areas of the Adriatic Sea to the high mountains Orjen, and Rumija and has a Mediterranean climate. In this are there the municipality of Herceg Novi, Kotor, Tivat, Budva, Bar and Ulcinj. Its population is about 145,000 inhabitants. This region comprises approximately 11.5% of the total area of Montenegro; it is home to about 23% of the population. Coastal region consists of the development zone of Boka Bay, central and southern coast. This region is relatively densely populated with businesses based on tertiary activities. GDP per capita is 3,600 Euros, which is slightly lower than in the central region and twice the GDP per capita in the northern region.

Most of the activity takes place along the coast linearly. In this area investment is of high pressure, which also causes uncontrolled development. The present spatial structures and conditions in the coastal area request appropriate organization of space and the management



of the future spatial development. Cessation of industrial activities will provide the potential for the creation of new tourist zones in the coastal area. These locations include: complex behind Krasic in Tivat, at Luštica - Pristan, Cape Trašte near Bigovo, the Flower Island, Platamune, Maljevik / Black Cape, part of the Port Bar, Volujac, Valdanos, Karaula on Bojana, Mamula, Lower and Upper Arzu, Ada Bojana, Solana Ulcinj, Exportbilje in Risan, warehouse in Zelenica. The development of tourist accommodation on the coast should be carefully planned, because the carrying capacity of the municipalities in the region have almost exhausted. The number of tourists in the high season of July and August can produce negative effects, such as overloading of transport infrastructure, congested due to lack of parking space, water shortages, polluted beaches, etc.

Development of tourist accommodation will be focused on: the area of the municipality of Ulcinj, on the locations Valdanos, Velika plaza with its hinterland, as well as Solana and Ada Bojana respecting the principles of sustainable development and natural values. The area of the municipality of Herceg Novi, mainly in locations, Kobila, Njivice Savina, Meljine-Lalovina, Zelenika, Kumbor, Baosici, Arza-Mirište-Žanjice, and Lustica. The area of Tivat, mainly in locations Pržno-Blue Horizons, Zupa and Boni i, revitalizing rural areas provided adequate planning. The area of Kotor, the locations Rtac (Risan), Raškov hill (Ljuta), on land in the open in Bigovo at locations above the cliffs of Žukotrlica to Terstena in the Lower Grbalj, Perast, Gornji Stoliv. The area of Budva, Becici on location, Kamenovo-Milo er Lucica, Buljarice, Jaz and revitalized villages in Paštrovi i. The area of the municipality of Bar, locations anj Veliki Pijesak, Utjeha and Maljevik. Tourist area of coastal mountains, has the potential for additional tourism such as hiking, climbing, mountain biking, and ski tourism (Subra on Orijen and Ivanova korita- Lov en).

One of the main problems is the large seasonal anthropogenic area, emphasized by narrow coastal strip and its weak communication link with the hinterland. Coastal region is known for illegal construction. Development intensity in some parts but it has all characteristics of so called "Walling" of the shore. If it keeps this intensity, it will lead to the final loss of attractiveness of coastal areas. (Jovicic, 2010)

2. Central (Central) area covers an area that edges mountains Orjen, and Rumija in the southwest and high mountain ranges of Durmitor, Bjelasice, Komova and Prokletija in the northeast and east. It has a continental climate in the lower regions of the mountains, and on the edges. In the lower regions it occasionally snows but lasts for short time while in the upland areas and reserves it stays for more than 4 months a year. This area includes the towns of Podgorica, Cetinje, Danilovgrad. This region accounts for about 35% of the total area of Montenegro and is home to about 45% of the population. Podgorica is also a major rail and road junction. The central region consists of development zones of Zeta and Bjelopavlicka plane, Niksic and Cetinje field, resulting in the most impressive lowland area surrounded by karst areas. The present spatial structures and conditions in the Central area need appropriate organization of space in light of the restructuring of the economy and the pressure on agricultural land.

In the Central region specific segments of tourism such as cultural and religious have great potential. Problems arise because of the widespread transit and excursion tourism in the area. Transit and excursion tourism in the Central region, especially in Cetinje and the area around Lake Skadar are to be completed by other forms of tourism, leading to prolonged stay of tourists in the region. Throughout the region, religious tourism is to be developed further (Ostrog Monastery, Cetinje monastery, monasteries and churches in the archipelago and the Skadar Lake, etc..) (Ljesevic, 2012).

Central region faces a number of major development and spatial-ecological problems. Population migration and deagrarisation are characteristics of this region, and they are in the rural areas of the northwestern part. It can be said that the depopulation and land

reclamation issue are present in all parts of rural areas, with the exception of the Zeta plain, and on the other hand, during a vigorous process of the migration of the population in Podgorica, as well as the growth of suburbs.

3. The northern region consists of the area of the mountain massif of Durmitor, Bjelasice, Komova and Prokletija. The climate is mountainous with mild summers and cold, harsh winters. Snow remains longer than 4 months a year. There are Bijelo Polje, Pluzine, Savnik, Zabljak, Pljevlja, Mojkovac, Rozaje, Berane, Kolasin, Andrejevica and Plav towns. This area of about 53.5% of the total area of Montenegro has about 32% of the population. The northern region consists of the development zones formed in the valleys of the Lim, and ehotina, Tara, Piva and Ibar rivers with mountainous areas. Even though agriculture employs a significant portion of the population, underdeveloped, and other natural resources are not sufficiently activated.

Concentrations of population are in several urban centers formed in the valleys of these rivers. GDP per capita is 1,800 Euros which is much smaller than in the other two regions. (Monographies Podgorica 2010) The present spatial structures and conditions in the northern region requires a spatial orientation which offers development inputs that will mitigate emigration processes, taking into account the protection of the environment. The specific role of mountain tourism is primarily to ensure the creation of possible additional activities for income generation and employment, strengthening rural development, avoiding further displacement and protection of, so called "Cultural landscape". We need to relate the development of tourism in Central and Northern region with other sectors of the rural economy, particularly in agriculture, food processing and handicrafts. This involves proper protection of the environment, architecture, landscaping, urban planning, etc.. It should provide protection from uncontrolled development, as it is happening now in Durmitor. (Final draft, 2006)

During the planning ski tourism development should take into account the negative effects of global warming, which will, presumably, be a limiting factor in the development of this type of tourism. Based on the aforementioned criteria, for the mountain tourism development there are the following tourist rural areas: Durmitor, Bjelasica and Komovi Mora ke mountains, Prokletije between the rivers Tara and Cehotina. In the area of Durmitor and Sinjajevine should be specifically supported the development of the following segments: Ski tourism, focusing on the slopes helmets, and edges (the Bukovici) as well as in Mali Stuoc, Savin Kuk, Javorova a, walking and hiking, mountain biking, hunting and fishing, for example. in Savnik, tourism, eg. in the Piva - Komarnica. This area offers a tour of the authentic natural resources, various biodiversity of mountains, rivers and lakes in the National Park "Durmitor", "Active & Extreme", for example. the NP "Durmitor" and Tara. The development of tourist accommodation should be careful because of the limited carrying capacity in this environmentally sensitive area. It is necessary to give priority to the development of medium and high standard than to accommodation of low standard.

In the Bjelasica and Komovi the development of the following segments will be promoted: Visiting of the authentic natural resources, rivers, lakes, and especially the National Park "Biogradska gora," Programs and excursion tours from view - the mountains, which are particularly popular for foreign tourists, various "wellness" programs to improve the physical and mental health by relaxing influence of climate and preserved nature, hiking, mountaineering, ski tourism, Active & Extreme, mountain biking. It is necessary to start from the functional differentiation of certain micro location on the premises of the area, (when new or amending existing spatial plans of municipalities) such as Marinkovac, Suvodo - Šiška (which includes attractive high chain Bjelasica with its highest tops), Jelovica Jezerine - Kljuc - Vranjak, Kolasin (which includes the town and a number of rural and cottage settlements in the region), to be considered for the development of tourism (Final draft,

2006). The development of tourist accommodation should be carefully planned in line with the carrying capacity. In the area of Montenegrin Prokletija the development of tourism in following segments should be promoted : Ski tourism, focusing on Cmiljevica - Turjak Ro aje - Haile - Štedin, Plav-Gusinje, Verusa-Mokro, tours through authentic natural resources with various biodiversity, rivers , lakes, hiking, climbing, mountain biking etc.

Moraca mountain area covers the area of spacious and typical chains near the rivers Mora a, Bijela, Gracanica and Mrtvica. Tourist valorization of attractive space is to be expected in the distant future, after the opening of the transport, infrastructure development and evaluation of hydropower potential of the Moraca, - area between the rivers Tara and Cehotina. Development is possible in the following segments: skiing and summer mountain tourism. It is necessary to give priority to the development of medium and high standard of accommodation.

Regional structure of Montenegro can be differentiated according to geographic distribution of natural conditions for the development, the existing links between the economy and the structure of the settlement, the general level of development and prospects for future development. These factors cause a certain regional homogeneity which differs in parts of Montenegro. Significant changes in political and economic terms that have taken place in this region, are the main reason for mitigation of regional disparities in development of Montenegro. (Monographies Podgorica 2010)

Production orientation in the most important regions in the Montenegrin agriculture is quite clearly differentiated. So, in the Coastal region of predominant agricultural activity and orientation is focused on the production of citrus fruits, early vegetables, olives, herbs, and seedlings of subtropical plants and greenhouse production:- in the central region there is production of continental fruits and vegetables, flowers, greenhouse production and collection of medicinal plants and wild fruits: and the Northern region production of milk, meat, potatoes, wool, fish farming and gathering berries. Commercial and other logic dictate that the use of comparative advantages should be carried out in the future, which means that in these regions would dominate existing or slightly modified production orientation. (Official Gazette of Montenegro, 2008)

Wherever possible, especially in the northern region, we need to stimulate the production of healthy food and organic agriculture. In the coastal region we need to stimulate the production and processing of Mediterranean crops. In the coastal region of the zone of intensive agriculture we need to save a minimum of 11.900 ha of which 8900 ha in Vladimirska and Ulcinjska fields and 3,000 ha in Grbaljska, Mr evo and Tivat fields.

In the Central region for intensive production we need to save about 40,500 ha, of which in Bjelopavlici plain, approximately 33,000 ha in Nikši field, Zupi and Grahovska area of 7,500 ha in the Northern region of intensive agriculture to 47,000 ha of the best land area of the river Lim and Ljubovinja, 19,000 ha in the area of Plav-Gusinje Basin and Ibar Valley area of 19,000 ha, and the area Pljevlja and Kosanica, about 9000 ha. For other agricultural production (healthy food and organic agriculture) we need to save land in the area of the upper canyon, Polimlja Plavsko - Gusinje valley and valley of the Ibar and ehotina.

Regionalization of Montenegro shows need for identification, development and implementation of specific tourism and recreation programs: traffic and telecommunications infrastructure, reconstruction of airports and water infrastructure, community infrastructure, improvement of the quality of tourism services; tourism development of rural hinterland; education for tourism, the implementation of equity, and institutional management, marketing and organizational restructuring of the tourism industry; specific forms of tourism in rural areas. (Jovicic, 2010)

### Conclusion

The work has theoretical and applied character which looks at the planning, legal and institutional frameworks of Montenegro. The authors in its conception first used a method of geosystem analysis, followed by statistical analysis. Administratively Montenegro is divided into 22 municipalities. Geographically Montenegro can be divided into three regions. In the Southern region of the zone of intensive agriculture a minimum of 11.900 ha of which 8900 ha in Vladimirsko and Ulcinjsko fields and 3,000 ha in Grbaljsko, Mr evo and Tivat fields should be saved. In the Central region for intensive production of 40,500 ha, of which Bjelopavlici plain, approximately 33,000 ha in Nikšić field, parish and Grahovsko area of 7,500 ha should be saved.

In the Northern region for intensive agricultural production should be set aside 47,000 ha of the best land. Of these, the area of the river Lim and Ljubovida, the 19,000 ha in the area of Plav-Gusinje Basin and Ibar Valley area of 19,000 ha, and the area Pljevlja and Kosanica, about 9000 ha. Significant changes in political and economic terms that have taken place in this region are the main solution for mitigation of regional disparities in the development of Montenegro.

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**CONNECTING AS A FACTOR OF SUCCESS FOR SME's FROM THE  
AGRICOMPLEX IN THE REPUBLIC OF MACEDONIA**

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**Abstract**

Entrepreneurial connecting is an efficient means for achieving a number of development opportunities for enterprises, and it can be motivated by market penetration, connection for market development, facilitation of purchase, sale, research/development, distribution, international trade and export, etc. The aim of this work is to indicate the need for entrepreneurial activities depending on current conditions of SMEs in the field of agri-complex that are the subject of this work. A survey research was conducted followed by data processing and analyses of data. The results obtained indicate that SMEs in the field of agri-complex mainly collaborate with one or a few enterprises, mainly based upon contracts, and they are not involved in a network of enterprises that collaborate; something that leads to weaker competence abilities of SMEs in the agri-complex on the domestic and foreign markets.

**Key words:** entrepreneurial connection, small and medium-sized enterprises, confidence, communication

**Introduction**

Small and medium-sized enterprises from the agri-complex should join their entrepreneurial strengths by connections, primarily in view of (Kostadinov, 2007): achievement of economies of scale, increased competitiveness in domestic and international markets, encouraging the possibility of innovation and commercialization of new products and services, increased pace of innovation, appearance on new markets, increased export, creation of new share capital or reduction of costs. Connecting enables enterprises to reduce uncertainties and risks, use of geographical differences and complementary expertise and efficient coordination and ability to meet the needs of major customers or simpler supplier structure. It also affects the efficiency and effectiveness of the enterprise and with it the decisions for joint ventures as well as the exchange of information between enterprises. Basically, the reasons for connecting could be defined aiming at (Rigsbee, 1999):

- Achievement of economies of scale and economy of related products,
- Achievement of the knowledge economy,
- Reducing of transaction costs,
- Internationalization.

Companies that intend to achieve economies of scale, economies of related products and knowledge economy expand their horizontal boundaries and connect horizontally. Those enterprises that want to lower the transaction costs and replace vertical integration would expand their vertical boundaries and would be linked vertically, while enterprises that want to connect in view of expanding their activities across the borders of their country (to internationalize) would search connecting both horizontally and vertically.

Entrepreneurial connecting is an effective tool for reaching numerous developmental opportunities for small and medium-sized enterprises from the agri-complex (Besanko et al., 2004):

- *Connecting in view of market penetration.* With this strategy, enterprises strive to strengthen and improve existing activities. Connecting helps participants to lower transaction costs (by rationalization and coordination, utilizing economies of scale and multiple strengthening of the market share),
- *Connecting in view of market development.* Cooperation in the area of market development often occurs when companies identify enterprises with complementary skills or products. Connecting is a means for entering new markets through existing distribution channels and exchange of resources,
- *Connecting in view of diversification.* The strategy of cooperation for accessing new markets with new products is closely related to connecting. Sometimes connecting with enterprises is a precondition for the accomplishment of certain activities. Often, companies must seek partners in foreign markets to meet certain governmental trade regulations or to form a consortium (multinational) in order to run in the public sector tenders.

It is possible to identify numerous areas of cooperation (Kreps, 1990):

- Procurement,
- Sale,
- Research and development,
- Marketing,
- Development of human resources,
- Production,
- Logistics,
- Distribution,
- Administration and management of development and training,
- International trade and development, etc.

Given the intensity of the connecting between the two extremities - unrelated market participation and formal association in a joint company – there are different types of connections (Deakins and Freel, 2010):

- Implicit contracts and long-term relationships,
- Agreement on teamwork,
- License agreement,
- Entrepreneurial business network,
- Strategic alliance,
- Joint venture.

The following key factors for success in entrepreneurial connecting can be indicated (Kostadinov, 2011):

- Mutual trust,
- Tolerance,
- Willingness to cooperate,
- Commitment,
- Mutuality (reciprocity),
- Communication,

- etc.

### Material and methods

Since a complete survey of the matter which is the aim of this research (the level of connecting/network of SMEs from the agri-complex) is practically impossible (a large number of SMEs in the agribusiness combined with the feelings of reluctance among some of them to take part in this survey) a partial survey was conducted. It is characteristic for this method of research that instead of the statistical body only a portion of it is examined, and the results of that study are expanded and are considered to be valid for the whole phenomenon. This method is also called the *method of selective* or *representative study*. In this research a random sample was applied, which is basically a sample containing randomly selected units of the statistical body lacking considerable representativeness. In this case a sample of 50 SMEs in the agri-complex was randomly selected whose owners were willing to participate in the survey. The assumption is that - despite the low level of representativeness - this sample can allow getting a proper picture of the current state of entrepreneurial connecting of SMEs from the agri-complex.

During the work several methods commonly used in economic analysis were applied, especially the method of generalization and specialization, the method of induction and deduction, the statistical method and the comparative method.

### Results and Discussion

In this survey respondents were offered a set of statements that are important for understanding the network of SMEs from the agri-complexes in the Republic of Macedonia valued at five-level scale, from 1 - *Strongly disagree* to 5 - *Strongly agree* (Table 1).

Table 1. Evaluation of the statements regarding the company networking.

Statement	Strongly disagree (%)	Partly disagree (%)	Neutral position (%)	Partly agree (%)	Strongly agree (%)
The company is associated with one or more companies through contracts	2.0	4.0	4.0	26.0	64.0
The company cooperates with one or several other businesses	4.0	0.0	0.0	38.0	58.0
The company is involved in a business network of enterprises that cooperate in areas such as purchasing, sales, distribution, research, etc.	84.0	6.0	4.0	0.0	6.0
The company operates completely alone and is not substantially related to other enterprises	2.0	2.0	2.0	8.0	86.0

Source: own calculations based on the results of the survey.

In order to get a focused picture of the networking of the enterprises in the agri-complex the

claims shown above were analyzed and synthesized in the following categories: *disagreement* (strongly disagree and partly disagree) and *agreement* (agree and strongly agree) (Table 2)

Table 2. Agreement/Disagreement among respondents regarding offered statements about company networking.

<b>Statement</b>	<b>Agreement %</b>	<b>Disagreement %</b>
The company is associated with one or more companies through contracts	6.0	90.0
The company cooperates with one or several other businesses	4.0	96.0
The company is involved in a business network of enterprises that cooperate in areas such as purchasing, sales, distribution, research, etc.	90.0	6.0
The company operates completely alone and is not substantially related to other enterprises	4.0	92.0

Source: own calculations based on the results of the survey.

Connection of enterprises with other enterprises from the agribusiness and other formal and informal actors and networks is one of the key factors for successful growth and development of the company. This research has shown that generally enterprises from the agri-complex in the Republic of Macedonia are not willing to form networks and cooperate with a number of partners. Cooperation between enterprises develops trust, reciprocity and related soft factors affecting the growth and development of the company. Enterprises from the agri-complex mainly work with one or several companies predominantly based on contracts, they are not involved in networks of enterprises that cooperate and entrepreneurs/owners mainly work alone and are not significantly associated with other companies. This situation not only is infertile field for the development of trust for long-term cooperation between enterprises, but decreases the efficiency and effectiveness of the development of existing clusters in the agri-complex and adversely affects the formation and development of new clusters. It leads to reduced competitive ability of enterprises both at home and at international level.

As a recommendation in this regard more intensive and long-term support of the government and social partners should be considered regarding connecting of enterprises in networks, participation in international agribusiness fairs, assistance in organizing various types of presentations and happenings regarding opportunities and products from the agribusiness mainly connected with innovative design offers and different types of activities. Measures for support should mainly be directed towards strengthening of the competitive abilities of agribusinesses in the Republic of Macedonia rather than obsolete types of protection of agribusinesses.

### **Conclusion**

Small and medium-sized enterprises from the agri-complex should associate their power in entrepreneurial connecting, primarily in view of: achievement of economies of scale, increased competitiveness in the domestic and international markets, encouraging the possibility of innovation and commercialization of new products and services, increasing the speed of innovation, appearance on new markets, increasing export, creation of new share capital or reducing costs. Linking enables enterprises to reduce uncertainties and risks, make use of the geographical differences and complementary skills as well as more efficient



coordination and ability to meet the needs of major customers or simpler structure of suppliers.

Research has shown that enterprises from the agribusiness in the country generally are not willing to network and cooperate with a number of partners. Cooperation between enterprises develops trust, reciprocity and related soft factors affecting the growth and development of the company. Enterprises from the agribusiness mainly work with one or several companies on the basis of contracts, they are not involved in networks of enterprises that cooperate and entrepreneurs/owners mainly work individually, not significantly associated with other companies. This situation is not only infertile ground for the development of trust for long-term cooperation among enterprises but also reduces the efficiency and effectiveness of the development of existing clusters in the agribusiness and adversely affects the formation and development of new clusters in the agribusiness. It finally leads to reduced competitive ability of enterprises both at home and at international level.

In this regard consideration about measures to support various forms of connectivity between enterprises should be recommended; something that should particularly be aimed at strengthening of competitive abilities of agribusinesses avoiding obsolete types of protection of agribusinesses in the Republic of Macedonia.

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**STATE OF INTERNATIONALIZATION OF SME'S FROM THE AGRI-COMPLEX  
IN THE REPUBLIC OF MACEDONIA**

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**Abstract**

Internationalization as an expression used the process of involving in and intensifying international operations. It is used to describe the continuum that initiates with the first import activity or extra regional expansion (“domestic internationalization”) up to total globalization. It involves activities like export, licensing, franchising, managerial contracts, agreements key-on-hand, contracts for production/international pre-contracts, agreements for industrial cooperation, mergers, acquisitions, strategic alliances, etc.

The aim of this work is to obtain information about the conditions of the internationalization of SMEs in the field of agri-complex in the Republic of Macedonia. With that aim, a survey questionnaire was composed followed by a survey. Obtained raw data were processed and analyzed, which enabled gaining insight in those conditions.

Research data have indicated that SMEs in the agricomplex are in the initial phase of their internationalization.

**Key words:** internationalization, SME, agri-complex, cooperation, contracts

**Introduction**

Entrepreneurs and those operating in the agri-complex can not isolate themselves from the globalization of the economy. Each business trades within the global economy, which actually means acceptance of strategies that allow entrepreneurs to optimize opportunities. These strategies will depend on the available resources, key personnel, product type and nature of technology. It may mean adopting strategies for joint venture; adopting quality techniques for benchmarks as part of a network of companies in the supply chain; creating networks to share resources and information. Entrepreneurs in the agri-complex must think globally, even if they work only on local markets.

The processes of association, liberalization, deregulation and democratization do affect and will be affecting the economy of the Republic of Macedonia in the near future. Therefore, Macedonian entrepreneurs and SMEs in the agri-complex need to confront the principles of internationalization and to timely adjust their international activities accordingly (Kostadinov, 2007).

Although it should be recognized that most authors believe that internationalization is associated with larger companies, it is only partially true. Small and particularly medium-sized enterprises tend to be active in the phases of establishment and growth (Kostadinov, 2011). Internationalization appears as the only solution to this pursuit. The reason for this is simple (Chell, 2001): most small and medium-sized enterprises do not have wide ranges of

products, i.e. they are often mainly focused on a narrow range of goods. In order to achieve economies of scale, companies have to seek new markets.

There are factors that hinder and factors that promote the internationalization of small and medium enterprises in the agribusiness, and some of these factors are (Poglejen, 2000):

- Attention, dedicated to meeting the needs of consumers. Here, SMEs often have difficulties because many times they lack resources to meet market needs;
- Quality of products in foreign markets is sometimes higher than that on the domestic market, which - unsurprisingly - hinders internationalization;
- The characteristics of management and needed management coverage. Entrepreneurs and managers of agro SMEs compared to larger companies often have lower levels of education and are less successful in the work on foreign markets;
- International experience, obtaining information regarding foreign markets, as well as support for the internationalization by the state. Trade barriers between countries (both official and unofficial; for instance, the attitudes to the origin of goods) hinders the internationalization of enterprises. International experience and easier access to information about the conditions on foreign markets encourage internationalization.

Small and medium-sized enterprises from the agri-complex often have harder difficulties in the process of internationalization compared to large enterprises. The reasons for such difficulties are (Deakins and Freel, 2010): bureaucratic barriers, customs barriers, late payment, choice of a distributor as well as communication difficulties with foreign buyers. Therefore, there is need for mutual aid so that the emerging markets will display some new forms, for example, networking with smaller foreign enterprises or with an export consortium.

According to one study (OECD, 1997) small and medium-sized enterprises have the following characteristics in terms of internationalization:

- They lack strategic planning activities, and consequently usually undertake internationalization measures in less efficient ways;
- Strategy based upon evolution - the lack of strategy planning is often the results of absence of opportunities, so that SMEs often only make use of available opportunities. Such an approach is rather opportunistic or not proactive;
- Companies that pay more attention to planning often choose a combination of different strategic options;
- The tendency towards internationalization increases with the maturity of the enterprise. Maturity of the enterprise here is measured by meeting the goals on the domestic market;
- Some business functions are better internationalized than others;
- There is not a single strategy for success, which means that if companies do not have clear visions of their development they can not accurately determine the tools that would achieve the objectives. Each company that intends to be present on international markets should create its own strategy.

### Material and methods

This study was applied on a simple random sample, which basically represents a sample containing randomly selected units from the statistical body. For the purpose of this research a sample of 50 randomly selected SMEs in the agri-complex was chosen whose owners/entrepreneurs agreed to participate in the survey. It is assumed that this sample can allow adequate insight into the current state of internationalization of SMEs from the agribusiness.

Several methods commonly used in the economic analysis were applied in this study, especially the method of generalization and specialization, the method of induction and deduction, statistical methods and the comparative method.

### Results and Discussion

In this survey the respondents were offered a set of assertions that are important for understanding the internationalization of SMEs in the agri-complex in the Republic of Macedonia valued at five-level scale from 1 - *Strongly disagree* to 5 - *Strongly agree* (Table 1),

Table 1. Rating of the statements regarding internationalization.

Statement	Strongly disagree (%)	Partly disagree (%)	Neutral position (%)	Partly agree (%)	Strongly agree (%)
The company makes purchase from abroad	32.0	4.0	2.0	6.0	56.0
The company exports	50.0	6.0	0.0	10.0	34.0
The company makes direct investment abroad	88.0	0.0	6.0	2.0	4.0
The company deals abroad through foreign business partners	34.0	0.0	2.0	26.0	38.0
The company has been dealing internationally since its beginning	84.0	2.0	4.0	0.0	10.0
The company works exclusively on the domestic market	30.0	8.0	0.0	10.0	52.0
The company aims to enter new foreign markets	18.0	6.0	14.0	6.0	56.0

Source: own calculations based on the results of the survey.

In order to get a focused picture of the internationalization of enterprises from the agribusiness the above claims were analyzed and synthesized in the following categories: *disagreement* (*strongly disagree* and *partly disagree*) and *agreement* (*agree* and *strongly agree*) (Table 2).

Table 2. Disagreement/agreement of respondents with the offered statements regarding internationalization

<b>Statement</b>	<b>Agree (%)</b>	<b>Disagree (%)</b>
The company makes purchase from abroad	36.0	62.0
The company exports	56.0	44.0
The company makes direct investment abroad	88.0	6.0
The company has been dealing abroad through foreign business partners	34.0	64.0
The company deals internationally since its beginning	86.0	10.0
The company works exclusively on the domestic market	38.0	62.0
The company aims to enter new foreign markets	24.0	62.0

Source: own calculations based on the results of the survey.

Internationalization begins with simple random or intentional connections in the field of import and export and continues with more complex forms of vertical and horizontal linkages and networking with other companies. This analysis indicated that enterprises from the agri-complex are still in their initial stages of internationalization. SMEs from the agri-complex are not very prone to export, while the propensity to import is somewhat higher. The tendency towards direct investment is negligible. Enterprises do not have the inclination to work abroad through overseas business partners. A negligible number of companies have been active internationally from the outset and many companies prefer to work exclusively on the domestic market. However, the sign of emphasized internationalization was observed. The fact that many entrepreneurs have set entering new foreign markets as a strategic goal is encouraging.

In terms of encouraging the internationalization of enterprises from the agri-complex in the Republic of Macedonia, campaigns can recommend to promote and encourage the internationalization of enterprises by increasing their competitive ability of entering new international markets. Then, the campaign should be supported by the educational system through general and specialized training for internationalization of businesses in the agri-sector. However, financial aid related to the process of internationalization should not be neglected. The state, through various mechanisms, should continue to financially participate in the development of agri-complex as well as facilitate the access to European funds aimed at agriculture and rural development.

### **Conclusion**

Entrepreneurs and those operating in the agribusiness cannot isolate themselves from the globalization of the economy. Each business trades within the global economy, which actually means acceptance of strategies that allow entrepreneurs to optimize opportunities. These strategies will depend on the available resources, key personnel, product type and nature of technology. internationalization may mean adopting strategies for joint venture, adoption of quality techniques for benchmarks as part of a network for companies in the supply chain; creating networks to share resources and information. Entrepreneurs in the agribusiness must think globally, even if they work only on local markets.

This research has shown that enterprises from the agribusiness in the Republic of Macedonia are still in the initial stages of their internationalization. SMEs from the agri-complex are not very prone to export, while the propensity to import is somewhat higher. The tendency towards direct investment is negligible. Enterprises do not have the inclination to work abroad through foreign business partners. A negligible number of companies have been active internationally from the outset and many companies prefer to work exclusively on the domestic market. However, what is encouraging regarding the higher level of internationalization is that many entrepreneurs have set the strategic goal of entering new foreign markets. The state should continue to financially support the development of the agri-complex and facilitate access to new European funds for agriculture and rural development through various mechanisms.

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**DETERMINATION OF AGRI-ENVIRONMENTAL SUPPORTING RATES TO PROTECT BIODIVERSITY OF INDIGENOUS SHEEP BREEDS**

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**Abstract**

The main objective of this paper is to determine the supporting rates for indigenous sheep breeds in context of the agri-environmental policy measures in the Republic of Macedonia. Agri-environmental measures can provide different level of preservation of livestock biodiversity, contribute at building higher nature value farming systems and ensure sustainability of low input breeding systems. The method used is based on partial budgeting, as a criterion framework used to compare the costs and the benefits arising from choosing different sheep breeds (three autochthonous sheep breeds: Karakachanian, Sharplaninian and Ovchepolian, as opposed to the usual practise). Thus, all aspects of farm profits, as well as the matching variable and fixed costs that remain unchanged are excluded. This procedure emphasizes the changes in income and costs that result from rearing alternative breed and enables computation of the financial supporting rate, as upper ceiling compensating the economic loss of the farmer. Moreover, the supporting rates for different sheep breeds are adjusted in terms of endangerment gradation and geographical distribution, using fitted coefficients. Support rates differ among breeds and also among output alternatives (cheese finalisation i.e. milk sales). The highest support rate was estimated for Karakachanian sheep (3,675 MKD i.e. 2,610 MKD), followed by Ovchepolian sheep (2,067 MKD i.e. 1,578 MKD) while the lowest rate was noted for Sharplaninian sheep (1,723 MKD i.e. 1,315 MKD). The results and the analysis revealed variations of the supporting rates. These aspects should be taken into consideration for further validation of the agri-environmental measures.

**Key words:** agro-environmental measures, Karakachanian, Ovchepolian, Sharplaninian, supporting rates.

**Introduction**

Agri-environmental measures (AEM) can provide different level of preservation of livestock biodiversity, contribute at building higher nature value farming systems and ensure sustainability of low input breeding systems. These measures support payments to farmers in return for implementing agri-environmental commitments that involve more than the application of usual good farming practice (COM, 2005). The main objectives of these measures are to diminish the environmental risks caused by agriculture and preserve the landscape (*ibid*). Therefore the mentioned measures include general directions such as: reduction of inputs, extensification of livestock production, farming of local breeds of animals that are highly adapted to the conditions of breeding and biodiversity conservation (Uthes *et al*, 2007). The participation in these programs is optional and voluntary, envisaging certain remuneration to the farmers for their commitment. These measures tend to compensate the most profitable use of land as the essential production factor and “payment levels have to be set sufficiently high to attract farmers to join schemes while avoiding over-compensation” (COM, 2005), thus requiring calculation of appropriate support rates.

Some specific conditions, next to biodiversity conservation and management of genetic resources, must be fulfilled in order for AEM to be realized. Namely, AEM can be provided for “local breeds indigenous to the area and in danger of being lost to farming” (Commission Regulation, 1257/1999) if the breeds significantly contribute to maintenance of the local environment and typical breeding systems in the country. Eligibility of local breeds for inclusion in the appropriate payment structure are defined in Commission Regulation (EC) No. 817/2004 where the threshold of each population is determined. A number of breeding females from each species, beneath which a breed is considered to be endangered and which are included in recognized register, represent the main threshold parameter. According to this regulation the thresholds are 7.500 for cattle, 10.000 for sheep, 10.000 for goats, 5.000 for equidae, 15.000 for pigs and 25 000 for avian species (Commission Regulation (EC) No. 817/2004).

The breeding of rare local breeds indigenous to the area helps maintain the generic diversity, but also indirectly contributes to preserving the landscape (COM, 2005). For the farmers point of view, it is more profitable and convenient to rear more yielding and improved breeds, hence deepening the endangerment.

Sheep production systems (extensive and intensive) are generally connected to climatic conditions, economic characteristics in the country and planned size of the farm. The specific climatic conditions and landscape in as well long tradition are ideal for development of sheep industry. In the Republic of Macedonia extensive semi-nomadic sheep breeding system is dominant, although recently there have been attempts to intensify production in some new sheep farms. From mid-May to early November sheep are in the highland pastures and during the rest of the year in the winter pastures. Technology in most farms is traditional, meaning lambing once a season (January-February), suckling period of lambs is until March-April and lactation period is to mid-July.

The main objective of this paper is to determine the supporting rates for indigenous sheep breeds in context of the agri-environmental policy measures in the Republic of Macedonia.

### **Materials and methods**

The method used is based on partial budgeting, as a criterion framework used to compare the costs and the benefits arising from choosing different sheep breeds (three autochthonous sheep breeds: Karakachanian, Sharplaninian and Ovchepolian, as opposed to the usual practise).

All names for Karakachanian sheep (Karakachanka, Black-Vlahian, Sara-Krachanian, Kuco-Vlahian, Albano-Vlahian and Karatsaniko) are associated with the name of the breeders of this sheep ethnic minority Vlachs. This breed has no concrete breeding region and often is bred in the most extensive region of the Republic of Macedonia. It is very resistant, modest and adaptive in extensive breeding conditions, vital and energetic animal, with lowest milk yield (24-26 l) per lactation. The coat color is grey-black and brown-black, but some time pure white individuals appear. The head is small with well spirally developed horns in rams, most of ewes are hornless (7-10% are horned). The wool is rough, coarse and long (up to 26 cm). The average weight of ewes is up to 33 kg and of rams is up to 44 kg. Regarding the tail length this breed belongs to the group of short tailed sheep.

Name of Sharplaninian population is originated from his originally area of breeding mountain massive Shar-Planina which is located in the Western part of the country. But today area of breeding of this sheep population is North-west and central part of the R. Macedonia. As the main characteristic of this population is complete white pigmentation of head, ears and legs. The head is tidily small, rams have well developed horns and most of ewes are polled but



sometime can appear horned individuals. The average weight of ewes is up to 32.3 kg and of rams is up to 44.2 kg. Lactation period is on average 199 days with milk yield of 62.60 L per lactation (with variation from 61 up to 120 L). Today up to 30 % of sheep population in the Republic of Macedonia belongs to this sheep population.

The Ovchepolian sheep got his name from the Ovchepolian plateau, area of breeding of this population, which is localized in the Eastern part of the Republic of Macedonia. Typical represent of this population has always full or partial pigmented head. Head pigmentation is black or brown. Face pigmentation have a shapeless spot which spans up to horn root, both visage sides up to mouth. Head is tightened and long. Snout is always black pigmented sometimes even in the interior of the mouth. Horns are well developed in rams but some time can appear individuals without horns, ewes are always pooled. The average weight of rams is up to 45 kg (35-48kg) and of ewes is up to 36kg (25-48kg). Lactation period is on average 191 days with average milk yield of 72.49 L, with great variation in milk yield (38.74L-91.28L). Today is present on 2/3 of the territory of the Republic of Macedonia.

The breeds supported by agri-environmental measure are compared to an average usual practice sheep population (case farm constructed on the base of the average statistical data where are raised crosses of Ovchepolian and Shraplaninian population with other improved sheep breeds).

The calculation of premia is typically performed on the basis on cost incurred and income foregone for the participating farmer in the agri-environmental program; support rate calculations are taking into account the variable costs and the loss of potential income. This procedure emphasizes the changes in income and costs that result from rearing alternative breed and enables computation of the financial supporting rate, as upper ceiling compensating the economic loss of the farmer. The calculated premia is expected to be “considerably higher than existing ones that cover marginal costs or marginal income forgone only” (COM, 2005). Though payments calculation or the support level considers additional costs and income foregone, some EU countries use combination of these elements and some even consider the transaction costs (Krisciukaitiene et al, 2007)

The partial budgeting approach emphasizes the changes in income and costs that result from implementing a specific alternative. Thus, all aspects of farm profits that are unchanged are excluded. In general, the fixed costs are regarded as equal, and in this case therefore omitted, so change is foreseen only in the area of the variable specific costs and in the yield/producer price level. The partial budget is flexible enough, analyses the impact of the profit on a certain change and can be used for analyzing a number of important decisions as modifying production practice is (www, Penn State). The format of a partial budget varies depending on the specific needs for the calculation, but additional costs, reduced revenue, additional revenue and reduced costs are always included in a partial budget no matter what the layout or organizational methods may be (Kay *et al.*, 2008).

Standard data regarding the technological features of the usual practice breed (crosses of Ovchepolian and Shraplaninian population with other improved sheep breeds) and the rare indigenous breeds were taken into account, in terms of typical outputs, and inputs. The output prices were gathered through the State Statistical Office (SSO, www), while input prices through direct farmer contacts.

Population thresholds (number of breeding females) below which a breed is considered to be endangered for the purposes of incentive payments are specified. The categorisation of the coefficient of endangerment is presented in Table 1, in local sheep populations is observed different level of endangerment.

Table 1. Categorisation of the coefficient of endangerment

<b>Indigenous breed</b>	<b>Category</b>	<b>CE</b>
Karakachanian (K)	(1) Critical, <300 heads	1,20
Ovchepolian (O)	(5) Not endangered, 3000 heads	1,01
Sharplaninian (Sh)	(5) Not endangered, 3000 heads	1,01

Additionally the geographical distribution coefficient values are added in order to illustrate geographical distribution of the breed (table 2) (Kastelic et al., 2006). Only Ovchepolian is determined as local, while the other two indigenous breeds are also present in neighboring countries.

Table 2. Categorisation of the coefficient of geographical distribution

<b>Indigenous breed</b>	<b>Category</b>	<b>GD</b>
Karakachanian (K)	Regional (in neighbouring countries)	1,00
Ovchepolian (O)	Regional (only in Macedonia)	1,20
Sharplaninian (Sh)	Local (in neighbouring countries)	1,00

The theoretical calculation of indicative supporting rates for breeding autochthonous sheep breeds was further based on application of the calculation formula distribution (Kastelic et al., 2006):  $YS = \frac{E}{CE} \times G$ , where: YS = yearly support, E = economic loss, CE = coefficient of endangerment, GD = geographical distribution.

The conversion of these breeds estimated for the purpose of this paper into Livestock Units is as follows: Karakachanian (0.07), Sharplaninian and Ovchepolian (0.09).

### Results and discussion

According to presented data in this research, based on official data, highest level of endangerment is noted for Karakachanian population. Thos population is the most endangered with less than 300 ewes, placed under the higher category of critical endangerment. Ovchepolian and Sharplaninian are ranked in the fifth category, with low level of endangerment.

Sheep farmers typically produce cheese as more profitable and less risky alternative to selling sheep milk to dairies. However, for illustration and comparison purposes, the economic loss of rearing autochthonous sheep is calculated also for the case of milk sales (Table 3 and 4).

The Karakachanian breed produces milk only to satisfy the needs for the lambs, hence resulting into visibly lower output value than all other breeds. The output volume and prices differ among the different alternatives; ranging from 1523 MKD in the case of Karakachanian up to 6868 MKD in the case of the usual practice breed. Once the partial cost items that differ among the alternatives are taken into account (excluding those costs that remain unchanged regardless the alternative breed reared), the economic loss in the “cheese option” is estimated at 1706 MKD for the Sharplaninian and Ovchepolian breeds, i.e. 3063 MKD for the Karakachanian breed.

Table 3. Partial budget of economic loss in usual practice versus autochthonous sheep production, cheese output option

<b>Output values</b>	<b>UP</b>	<b>K</b>	<b>O</b>	<b>Sh</b>
Milk yield (kg/ewe)	70	26	50	50
Milk for lambs (kg)	15	26	20	20
Cheese from remaining milk (kg)	15.7	/	8.6	8.6
Cheese price (MKD/kg)	260	/	260	260
<b>Output value – cheese</b>	<b>4086</b>	<b>/</b>	<b>2229</b>	<b>2229</b>
Wool	2.5	1.5	1.7	1.7
Wool price (MKD/kg)	25	15	15	15
<b>Output value – wool</b>	<b>62.5</b>	<b>22.5</b>	<b>25.5</b>	<b>25.5</b>
Lamb (kg)	17	10	12	12
Lamb price (MKD/kg)	160	150	150	150
<b>Output value – lamb</b>	<b>2720</b>	<b>1500</b>	<b>1800</b>	<b>1800</b>
<b>Total output value</b>	<b>6868</b>	<b>1523</b>	<b>4055</b>	<b>4055</b>
<b>Partial differentiated costs (PDC) in MKD</b>				
Alfalfa hay (MKD)	880	800	800	800
Concentrate feed (MKD)	1800	1200	1500	1500
Cheese processing costs	1603	/	874	874
<b>Total PDCs</b>	<b>4283</b>	<b>2000</b>	<b>3174</b>	<b>3174</b>
Partial difference	2585	-2283	-1109	-1109
<b>Economic loss</b>		<b>-3063</b>	<b>-1706</b>	<b>-1706</b>

In the case of “raw milk” sales (Table 4), the output value is lower in all four alternatives, but also the cost value is lowered since the cheese processing costs are omitted. The output value is 1523 MKD for the Karakachanian breed, 2696 MKD for the Sharplaninian and Ovchepolian breeds, and 4378 MKD for the usual practice. Expectedly, the economic loss is lower and is estimated at 1302 MKD for the Sharplaninian and Ovchepolian breeds, i.e. 2175 MKD for the Karakachanian breed.

Table 4. Partial budget of economic loss in usual practice versus autochthonous sheep production, milk output option

<b>Output values</b>	<b>UP</b>	<b>K</b>	<b>O</b>	<b>Sh</b>
Milk yield (kg/ewe)	70	26	50	50
Milk for lambs (kg)	15	26	20	20
Milk for sale (kg)	55	/	30	30
Milk price (MKD/kg)	29	/	29	29
<b>Output value – milk</b>	<b>1595</b>	<b>/</b>	<b>870</b>	<b>870</b>
Wool	2.5	1.5	1.7	1.7
Wool price (MKD/kg)	25	15	15	15
<b>Output value – wool</b>	<b>62.5</b>	<b>22.5</b>	<b>25.5</b>	<b>25.5</b>
Lamb (kg)	17	10	12	12
Lamb price (MKD/kg)	160	150	150	150
<b>Output value – lamb</b>	<b>2720</b>	<b>1500</b>	<b>1800</b>	<b>1800</b>
<b>Total output value</b>	<b>4378</b>	<b>1523</b>	<b>2696</b>	<b>2696</b>
<b>Partial differentiated costs (PDC) in MKD</b>				
Alfalfa hay	880	800	800	800
Concentrate feed	1800	1200	1500	1500
<b>Total PDCs</b>	<b>2680</b>	<b>2000</b>	<b>2300</b>	<b>2300</b>
Partial difference	1698	-680	-380	-380
<b>Economic loss</b>		<b>-2175</b>	<b>-1302</b>	<b>-1302</b>

The support rates are product of the economic loss and the coefficients of endangerment and geographical distribution; in the first option (Table 5), the support rates range from 1723

MKD/head or 21533 MKD/LU for Sharplaninian to 3675 MKD/head or 52506 MKD/LU for Karakachanian. In the raw milk sales option (Table 6), the highest support rate was estimated for Karakachanian sheep with 2,610 MKD/head or 37286 MKD/LU, followed by Ovchepolian sheep 1,578 MKD/head or 17534 or 37286 MKD/LU, while the lowest rate was for Sharplaninian sheep 1,315 MKD/head or 16438 or 37286 MKD/LU.

Table 5. Support rates for indigenous sheep breeds, with cheese output option

Breed	Support rate per head (MKD)	Support rate per head (€)	Support rate per LU (MKD)	Support rate per LU (€)
Karakachanian	3675	59.76	52506	853.76
Ovchepolian	2067	33.61	22968	373.47
Sharplaninian	1723	28.01	21533	350.13

Table 6. Support rates for indigenous sheep breeds, with milk output option

Breed	Support rate per head (MKD)	Support rate per head (€)	Support rate per LU (MKD)	Support rate per LU (€)
Karakachanian	2610	42.44	37286	606.27
Ovchepolian	1578	25.66	17534	285.10
Sharplaninian	1315	21.38	16438	267.28

### Conclusions

The European experience is that the agri-environment measures are highly accepted by farmers, with a correspondingly high level of compliance. Having payment calculations in place, following the standard methods for estimation of the support rates, is of high importance in the processed of planning and projecting this type of measures.

In our research, we came to estimate that the support rates differ among breeds and also among output alternatives (cheese finalisation i.e. milk sales); The highest support rate was estimated for Karakachanian sheep (3,675 MKD i.e. 2,610 MKD), followed by Ovchepolian sheep (2,067 MKD i.e. 1,578 MKD) while the lowest rate was noted for Sharplaninian sheep (1,723 MKD i.e. 1,315 MKD). The results and the analysis revealed certain variations of the supporting rates, depending on the basis of calculating the output form. These aspects should be taken into consideration as range values when planning the agri-environmental measures. Defining and implementation of specific AEM for indigenous sheep populations in the future will represent solid base for their preservation that directly will provide conservation, characterization and promotion of animal genetic resources in order to protect livestock biodiversity.

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**THIEU LITCHI PRODUCTION IN THANH HA DISTRICT (HAI DUONG PROVINCE, VIETNAM): PRODUCERS AND EXTENSION SERVICES STRATEGIES**

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**Abstract**

The Thanh Ha district (Hải Duong province, North-Vietnam) has a long history of traditional Thanh Ha Thieu litchis cultivar production. However, the price paid to the farmers for this traditional cultivation has been reducing significantly for more than 10 years. In this communication, recent evolution of Thanh Ha Thieu litchis production and strategies established by producers in order to face the fall in price are studied and a diagnosis of the agricultural extension services involved in Thanh Ha Thieu litchi production is drawn up. Primary data were collected in 2012 through personal interviews and focus groups with multiple stakeholders, authorities and extension organisms and a survey of producers' households made with semi-structured questionnaires.

Substantial reduction of litchis production participation in total household incomes was revealed. In parallel, Thieu litchis plantations superficies fall down being converted in favor of new fruit species or in favor of other litchis varieties depending on the production area operating a diversification process. Moreover, progressive abandonment of not converted plantations is marked by plantations cares reduction.

In the past five years, all agricultural extension sessions about litchis production have been initiated and organized by the authorities at province and district level collaborating with input supply private companies. Various inefficiency problems are highlighted such as disequilibrium in training session access, relative trainings uselessness or mismatch between producers' training demand and offer.

**Key words:** Vietnam, Thieu litchi, Diversification, Extension services, Decentralization.

**Introduction**

In 1986, the Vietnamese Communist Party, head of The Socialist Republic of Vietnam, launched a new economic policy called “Đổi Mới”. The objective was to convert planned economy into a socialist-oriented market economy operating many reforms. In agriculture, one attends the individual attribution of arable lands to farmers' households and the liberalization of activities between agro-food supply chain stakeholders (Pham Ngoc, 2007). A family agriculture is set up with the appearance of farmers' technical-economic autonomy (Vu Dinh, 2003).

Within this context, the State progressively proceeded to the retirement, dismantlement, reconstruction and decentralization of the agricultural support services previously integrated in State cooperatives. Great extent and speed of the reforms constitute a success. Nevertheless, reforms led to important support services access inequalities based on production speculations, regions and incomes and to creation of institutional gaps in support services provision (Lavigne Delville *et al.*, 2007). Moreover, support services decentralization process differs from one province to another depending on the interpretation

done of unclear competence transfer (Hoang Anh, 2006) and according to the private sector importance in support services provision (Fforde *et al.*, 2003).

This communication focuses on agricultural extension services involved in fresh Thieu litchi production in Thanh Ha district (Hai Duong province, North-Vietnam) in Doi-Moi reforms context.

In 2010, labor population in Thanh Ha, represented 56% of total (153645 inhabitants) of which 73% worked in agriculture, forestry and fishery sector (Thanh Ha Statistics Office, 2011).

The district presents adequate soils and climate for litchis production with one dry and fresh season from October to April and one wet and hot season from May to September. Litchi production is a risky culture, sensitive to climate variation and with high seasonality character due to its unique annual short harvest period (15 days from the end of April and the beginning of August in function of early or late cultivar varieties).

Especially the Thanh Ha Thieu litchi, late harvest variety, is historically recognized in all Vietnam for its organoleptic qualities and the traditional know-how of its producers (Tham Tram, 2005). Before 1993, plantations were limited to familial gardens. From that year, in the framework of Doi Moi reforms, farmers started to convert rice cultures into litchi plantations. At the beginning, this conversion allowed farmers to increase substantially their incomes and living standards. However, the price paid to the farmers for this cultivation has been reducing significantly for more than 10 years (from 14000 VND/kg in 1997 to 2000 VND/kg in 2011) with similar annual yields which is threatening the future of this production.

Municipalities in Thanh Ha district can be classified in four different principal litchis production areas in function of produced litchis quality (first, second or third class), cultivars structure (early, hybrid or Thieu litchis dominance), garden plantation ages (> 50 years, 35-45 years, 15-25 years) and conversion years from rice to litchis plantations (1995-1996, 1996-2000, 2000-2001). Litchis production areas characteristics are presented in table 1.

Table 3. Litchis production areas classification according to litchis quality, cultivar structure, garden plantation ages and conversion years.

Production area	Municipalities	Litchis quality	Principal cultivar	Garden plantation ages	Conversion years
A	Thanh Son, Thanh Thuy, Thanh Xa, Thanh Khe and Thanh Xuan	First class	Thieu litchis	> 50 years	1995-1996
B	Phuong Hoang, An Luong, Thanh Hai, Tan An, Tien Tien, Thanh Ha Town, Cam Che, Lien Mac, Thanh An, Thanh Lang	Second class	Thieu litchis	35-45 years	1996-2000
C	Hop Duc, Truong Thanh, Thanh Binh, Thanh Hong, Thanh Cuong, Vinh Lap	Second class	Early litchis	35-45 years	1996-2000
D	Quyet Thang, Hong Lac, Tan Yen, Viet Hong	Third class	Thieu litchis	15-25 years	2000-2001

Sources: own elaboration; Lavigne Delville *et al.*, 2004; Nguyen Tien, 2005; Vu Dinh, 2006.

The first objective of this paper is to identify recent evolution of litchis production and strategies established by producers in order to face the fall in price and improve their incomes.

The second objective is to draw up a diagnosis of the litchi production-related agricultural extension services structure.

## Materials and methods

After literature researches and secondary data analyzes, important fieldwork has been done in 2012 in order to collect primary data. Fieldwork focused on three municipalities representative of the A, B and C production areas: Thanh Son, Thanh Binh, Cam Che. The fieldwork did not focus on the D production area because litchi production in this area is historically insignificant.

First fieldwork consisted in field observation in the study area.

Secondly preliminary and complementary interviews were made with multiple stakeholders through personalized open interview guides. This permitted to obtain an overview of the litchi production operations, the stakeholders involved in extension services so as links between all actors.

Thirdly, a survey of 30 producer households has been carried out in the three aforementioned municipalities. In each commune, 10 households were interviewed. The 30 interviewed families were classified according to their litchis plantations size (< 2 sao<sup>32</sup> / between 2 and 7 sao / > 7sao; 1 sao = 360 m<sup>2</sup>). 3 or 4 families of each size class were interrogated in each commune. The questionnaire was semi-structured. First part of the questionnaire (closed questions) concerns ultimate litchi production tendencies and households' characterization. Second part (closed and open-ended questions) focuses on agricultural services characterization. This survey allows assessing of strengths and weaknesses of the extension services from the recipients' perspective.

## Results and discussion

### Agriculture importance in surveyed households' activities

The 30 surveyed households' total population is 121 persons, representing an average of 4 members per household. The mean age is 37 years. On average, 2.9 persons per household practice remunerated activities representing 87 persons. Among those working people, 42% perform only agricultural labor, 30% combine agricultural work with another remunerated activity and 28% do not participate in agricultural activities. In 2011, agricultural incomes and more specifically litchis production incomes represented respectively 58 % and 32% of total households' incomes.

For 83% of households, perennial fruit cropping constitute the principal agricultural income-generating activity. For the remaining 17%, this is animal husbandry that constitutes the principal agricultural income-generating activity. In Thanh Son, fruit cropping constitute the exclusive agricultural income-remunerated activity. In Cam Che and Thanh Binh, respectively, 45% and 30% of households have fruit cropping as exclusive agricultural income-remunerated activity, 22% and 0% combine fruit cropping only with animal husbandry, 33% and 60% combine fruit cropping with animal husbandry and food crops and 0% and 10% combine fruit cropping only with food crops.

### Recent litchis production evolution

Below there are some of the main results of the survey carried out.

A. In 2012, the mean size of owned agricultural land is of 12.1 sao<sup>33</sup> per household with a mean of 11.6 sao in Thanh Ha, 13.5 sao in Cam Che and 11.3 sao in Thanh Binh. In Thanh Ha, Cam Che and Thanh Binh the mean size of owned litchis plantations is

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<sup>32</sup> Vietnamese unit of area, 1 sao = 360 m<sup>2</sup>.

<sup>33</sup> Vietnamese unit of area, 1 sao = 360 m<sup>2</sup>.



respectively 5.7 sao, 5.5 sao and 8.9 sao. Between 2007 and 2012, Thieu litchis plantations superficies have fallen down being converted in favor of new fruit species in Thanh Son and Cam Che or in favor of early litchis varieties in Thanh Binh.

In Thanh Son and Cam Che, Thieu litchis plantation superficies per household have decreased by, respectively, 5.9 sao (51% less from 11.6 to 5.7 sao) and 3.6 sao (40% less from 9.1 to 5.5 sao) between 2005 and 2012. Thieu litchis plantations conversion have been mostly done in favor of kumquats superficies in Thanh Son (more 5 sao from 2005 to 2011) and in favor of guavas superficies in Cam Che (more 2.7 sao from 2005 to 2011). Proceeding to such a production diversification permit to households incomes not to depend almost exclusively of litchis production that constitutes a risky culture (one short annual harvest period and high climate variation sensibility) with low selling prices. However such a diversification means a great producers know-how lost and the very high quality Thieu litchis superficies diminution.

In Thanh Binh, according to the Municipality Agriculture Office Director, litchis plantations superficies remained constant since 2000 but the cultivar structure has changed. Thieu litchis superficies have fallen down from more than 70% of total litchis superficies to less than 50% in favor of early litchis varieties that now cover more than 50% of total litchis superficies. However, during the fieldwork, some Thieu litchis production parcels in Thanh Binh were observed in conversion phase in favor of guavas. That could be the beginning of a diversification process similar to the aforementioned municipalities Thanh Ha and Cam Che.

B. In parallel, substantial reduction of litchis production participation in total household incomes was revealed. This reduction comes from a diminution of both agricultural activities incomes in total households incomes and litchi production incomes diminution in total agricultural households incomes. The households incomes structure for the 2007 and 2011 years is presented in table 2. Even if agricultural activities still represent the major incomes source, agricultural incomes decrease from 67% to 58% of total incomes between 2007 and 2011 in parallel with non-agricultural practices share increase. In the same time lap, litchis production incomes decreased from 75% to 55% of agricultural incomes (from 50% to 32% of total incomes).

Table 2. Households income structure for the 2007 and 2011 years.

Year	2007	2011
Total incomes	100%	100%
Agricultural incomes	67%	58%
<i>Litchis production</i>	75%	55%
<i>Other productions</i>	25%	46.5%
Non-agricultural incomes	33%	42%

Source: survey results

In 2007, litchis production represented more than 60% of total incomes for 50% of the households. Between 2007 and 2011, this percentage falls down from 50% to 7%.

C. On top of that, progressive abandonment of not converted plantations is marked by plantations cares reduction. This can first be observed in many litchis plantations invaded by weeds and where trees are not pruned anymore. Moreover, 27% of producers say they spent much less time to litchis plantations cares in 2011 than in 2007; 27% less time, 23% the same time, 13% more time and 10% much more time. All producers that spend more or much more time to plantations cares come from Thanh Binh. Producers explain this time diminution by a diminution of the time dedicated to harvesting, weeding and pruning.

### Diagnosis of Agricultural Extension

In the past five years, all agricultural extension meetings about litchis production have been initiated and organized by input supply private companies and by the authorities at province – Department of Agriculture and Rural Development and Center for Agricultural Extension – and district level – Agricultural Office and Center for Agricultural Extension District Unit. The role of the Department of Agriculture and Rural Development and of the Agricultural Office is limited to “state management” without getting involved in direct provision of extension services. They rely on the Centre for Agricultural Extension at province and district level to work on agricultural extension activities.

The Center for Agricultural Extension often also relies on private inputs supply companies in order to organize inputs application trainings. It seems that most of those trainings have more a business and publicity purpose than a real households’ empowerment purpose. It is possible to speculate that the provincial Center for Agricultural Extension has been partially privatized. Communal agricultural cooperatives sometimes help in the extension sessions organization but do not initiate them.

Extension services decentralization process seems to operate as a combination of both delegation (delegation of responsibilities within the state administrative system from province to district level) and deregulation process (delegation of task outside the state to private structures).

The mean of litchis production training session assistance is one per producer a year. However, not all but 50% of the producers have already assisted to one or more training sessions (from 1 to 6 a year) about litchis production. And only 13% of the surveyed producers share more than half of total training sessions’ participations. This shows an imbalance in access to training sessions.

For the year 2011 and until May 2012, the total number of training sessions’ participations was 32. And almost all training sessions (29 out of 32) concerned the application of inputs – fertilizers, pesticides, fungicides, growth regulators and herbicides. The remaining three training sessions were about VietGAP standards<sup>34</sup>.

Among the producers that have recently (in 2011 and until May 2012) attended training sessions about inputs application (15 out of 30), 13% and 47% consider training sessions as, respectively, totally useless or pretty useless while 27% consider them as pretty useful and 13% as very useful. On top of that, 19% of those producers say they never changed their practices after attending training sessions, 56% not often, 19% often and 6% very often. It’s also important to mention that 40% of those producers have already rejected invitations to attend training sessions. It reveals a tendency to consider training sessions as useless. The three producers that have attended to GAP standards training sessions say it was pretty useless and they did not change their production practices after training sessions attendance.

A mismatch between producers’ training demand and what is offered is also highlighted. Instead of inputs application training sessions, they are interested in training sessions about, in order of importance, litchis preservation techniques (40%) in order to extend the harvest period, diseases prevention techniques (20%), litchis drying techniques (10%) and VietGAP standards (10%).

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<sup>34</sup> Vietnamese Good Agricultural Practices standards (VietGAP standards) were developed in 2008 by the Ministry of Agriculture and Rural Development to promote high quality product supply chain. It refers to an official certification process used to insure that good agricultural practices are respected during production, harvesting and post-harvesting operations (MARD, 2008).

## Conclusion

In a first time, the work focuses on identification of the recent evolution of litchis production and the strategies established by producers in order to face the fall in price and improve their incomes. Thieu litchis plantations superficies fall down being converted in favor of new fruit species or in favor of other litchis varieties depending on the production area operating a diversification process. In parallel, a substantial reduction of litchis production participation in total household incomes was revealed. Moreover, progressive abandonment of not converted plantations is highlighted.

In a second time, the work draws up a diagnosis of the litchi production-related agricultural extension services structure. In the past five years, all agricultural extension meetings about litchis production has been initiated and organized by the authorities at province and district level collaborating with input supply private companies according to a delegation and deregulation process. Various inefficiency problems are highlighted such as disequilibrium in training session access, relative trainings uselessness or mismatch between training producers demand and offer. Authorities have to work in order to solve those problems.

This paper focuses exclusively on agricultural extension services involved in litchis production. In a further work, all agricultural support services at all supply chain levels considering all interconnections will be analyzed.

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**ECONOMIC EFFECT, EXPERIENCES AND EXPECTATIONS IN  
AGRICULTURAL COOPERATION IN THE REPUBLIC OF CROATIA**

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**Abstract**

Cooperatives have significant role in economic development and this paper is affirming cooperative philosophy and other ethical cooperatives values. Family farms in the Republic of Croatia are identified as basic and key factors in agriculture cooperative business. Those farms should recognize advantages, adjust to the market and accept changes and new knowledge. Cooperatives in the Republic of Croatia have a rich and long tradition, but with a very turbulent development. The first cooperatives were established on the Croatian territory more than 150 years ago and in some period they were very important economic institutions (Sudaric *et al.*, 2010a; 320). However, different economic and political conditions have redirected this development and have decreased cooperative activities. The aim of this paper is to present cooperative position in the Republic of Croatia and its potential in retrospective to the Ministry of Agriculture and Ministry of Labor and Entrepreneurship program implementation. New law regulation, different kind of subvention as well as cooperative system of global meaning in the Republic of Croatia is on the way to affirming and supporting this kind of business organization. This paper also contains survey research about opinions of agricultural producers about cooperative business as well as importance of cooperatives in joining to European cooperative society.

**Key words:** agricultural cooperatives, development, economy, Republic of Croatia

**Introduction**

Cooperatives in the Republic of Croatia have a long history. However, the period of socialism - when the principles of cooperative organization and business model tried to adopt the socialist central planning and management economy - today leaves specific negative elements and prevents the powerful development momentum of this sector in the Republic of Croatia (Mataga, 2009; 246). There are many other problems like legislation, administrative barriers, lack of information and misunderstandings of farmers that did not lead to the desired result.

Agricultural production in the Republic of Croatia is dispersed in a large number of family farms. Farms are not self-sufficient and enough competitive neither for domestic nor for European markets. Cooperatives generally combine purchases and sales for tens and hundreds of their members, cooperate, and represent rational organization, service, database, source of information for their business cooperators (Sudaric *et al.*, 2010b: 155).

The European Commission data show the importance of cooperatives in the European economy because nowadays in the European Union operate approximately 250,000 cooperatives with 163 million cooperative members, or every third citizen of the European Union is in a cooperative. Cooperatives employ nearly 5.4 million people<sup>1</sup>.

Family farms in the Republic of Croatia are defined as the most important subjects of agricultural structure or as contractors of Croatian agriculture. According to the analysis of ownership structure it can be seen that they are very small operators that cannot achieve the necessary amount, quality and supply continuity of agricultural product. They are also in disadvantaged situation in acquiring raw materials, applying modern technology, finalizing processing and sale of finished products.

According to the Farm Register, 192,173 farms are registered in 2013, of which 96% are family farms, and in smaller ratio are registered as crafts, trades, society groups or cooperatives. Those farms have commercial status and they are in the support production system and generate production for the market. Observing average size of farms registered in the Farm Register to the land possession, the largest farms are in the eastern part of Croatia. The Croatian farm size average is 5.9 ha, and only 4% of farms has land possession larger than 20 ha.

Croatian accession to the European Union and further adjustments to the European market will affect that existing agrarian structure. They will experience some changes reflected in the increased number of farms that will tend to economic size of land property, or on the other side to keep small businesses with mixed farming, and the implementation of additional economic activities. However, changes in agricultural structure will be reflected through the new empowerment of cooperative business, which has been accepted and which successfully operates in many countries of the European Union and wider.

The aim of this paper is to identify issues of Croatian agriculture and to identify the importance and potential of cooperatives for the overall economy through social and economic value of cooperatives.

### **Materials and methods**

In this paper, besides relevant domestic and foreign publications, we used data from the Croatian Cooperatives Union, Ministry of Agriculture, the Ministry of Economy, Labour and Entrepreneurship as well as empirical research conducted during the 2013 year. The sample was consisted of 117 farmers who expressed their views and opinions regarding the cooperative system in the Republic of Croatia. The entire questionnaire consisted of 12 questions of open-ended and closed type of questions divided into several groups, related to the type of agricultural production, the legal status of farms, ownership structure, and views of supporting and development of agricultural cooperatives in the Republic of Croatia. The sample consisted of randomly selected respondents - participants who deal with agricultural production from eastern Croatia. Respondents show their opinion on the five (5) negation and four (4) affirmation statements with whom they had to agree or disagree, and which are related to the support / lack of incentives for the cooperatives development in Croatia. Respondents' replies were measured by five stages Likert scale: 1 = strongly disagree, 2 = disagree, 3 = neither agree nor disagree, 4 = agree, 5 = strongly agree). In data processing we used standard deviation, sample variance, median, mod and mean. A chi-squared test, also referred to as chi-square test or  $\chi^2$  test, is any statistical hypothesis test in which the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true.

### **Results and discussion**

According to the Croatian Cooperatives Union in December 2010 in Croatia were active 1691 cooperatives, while in the 2009 existed 1125 cooperatives according to the final reports

of the Financial Agency (FINA). The largest number of cooperatives in 2009 was agricultural cooperatives with a share of 60.36% in the total number of cooperatives, followed by services cooperation (20.62%) and commercial cooperatives (9.60%) while the least were the savings and loan cooperatives (5.16%) and housing cooperatives, which accounted only 4.27% of the total number of cooperatives ([www.zadruga.hr](http://www.zadruga.hr)).

A cooperative as the movement develops according to the basic cooperative principles which are adjusted to the changes of society and the environment. The role of knowledge, information and education is a key element of the cooperative concept. Therefore, as part of this study is empirical research conducted of 117 random sample farmers who expressed their views and opinions regarding the cooperative system of Croatia. The aim of this survey was to explore and define the basic problems of the agricultural cooperatives development.

By education level, 15.4% of respondents had only completed primary school, 63.2% have a high school education, 18.8% had completed college or university level, 1.7% of respondents have a master's degree or PhD and one respondent (0.9%) is with no school. The average age of the respondent was 45.80 years. Among them 77.8% were men and 22.2% women. Ownership structure of respondents is in much more favorable position compared to the Croatian average. Specifically, 48.7% of the respondents have more than 10 ha, 27.4% of them had more than 3 ha, while only 10.3% had less than 1 ha farm.

Mostly, respondents were registered as family farms that have been registered in the Register of farmers (65%) and with a significantly smaller share as family farms that are not registered in the Register of farmers (22.2%). Worrying is the fact that only 1.7% of the randomly selected respondents is in agricultural cooperative. According to research, 43% of survey respondents believe that they are more successful as the individual producers rather than as members of the cooperative.

Table 1. Selected descriptive statistical indicators relating to the attitude statements.

Statement	Number of data	Mean	Median	Mode	Standard Deviation	Sample Variance
1.	117	3.55	4.00	5.00	1.24	1.53
2.	117	3.76	4.00	5.00	1.21	1.46
3.	117	3.90	4.00	5.00	1.10	1.21
4.	117	3.62	4.00	5.00	1.11	1.24
5.	117	3.40	4.00	5.00	1.29	1.66
6.	117	3.67	4.00	5.00	1.20	1.45
7.	117	3.91	4.00	5.00	1.13	1.27
8.	117	3.81	4.00	5.00	1.07	1.15
9.	117	4.20	4.00	5.00	1.04	1.08

*Source: own research*

Negation statements (1-5) were about fact that problems in the cooperatives development in Croatian are reflected in lack of information and farmers ignorance (Statement 1); mutual distrust and the past burden (Statement 2); lack of financial and organizational national support (Statement 3); inadequate legislation (Statement 4); investment problems and profit sharing (Statement 5).

Affirmation statements (6-9) were about easier way to obtain financial benefits and support (Statement 6); get better and faster information and help (Statement 7); get new knowledge and education (Statement 8); and interconnect and achieve better connectivity with the market (Statement 9).

Most of the respondents in the statements case, evaluated the analyzed elements of grade 4 or 5, but best evaluated were statement where respondents believe that the cooperative can be interconnected to achieve better correlation with the market, organization of production and distribution of goods (4.20) and that is the most important component of the cooperatives development.

The chi-square test with a significance level of  $p > 0.05$  indicates that problems in cooperatives development in Croatia reflects on the importance of the lack of information and farmers knowledge in mutual distrust, the lack of financial and organizational support, inadequate legislation and the problems about investments and income distributions which are significantly different from the normal distribution.

In Croatia, the number of cooperatives has increased which certainly favor the various programs and support in cooperative system. The responsible ministry for cooperative business is the Ministry of Economy, Labour and Entrepreneurship, with the exception of agricultural cooperatives where the Ministry of Agriculture still has the highest impact. The Ministry of Agriculture has encouraged the work of agricultural cooperatives in three sub-programs:

- Establishment of new agriculture cooperatives
- Development of agriculture cooperatives
- Co-financing professional managers in agricultural cooperatives

The aim of the program was to encourage the establishment and development of cooperatives, and to connect small producers into cooperatives in order to create cooperative products. Co-financing refers to agricultural cooperatives registered in the Croatian Cooperatives Association and having 7 or more people in cooperative business as well as has defined agricultural production.

Most respondents (62.4%) agree with the statement that the government encourages the establishment of cooperatives and cooperative development projects & cooperative systems, in accordance with the economic and social development strategy, which is connected with the fact that 64.9% of respondents were familiar with measures to encourage cooperatives development in the Republic of Croatia.

According to the draft version of the Rural Development Program in the Republic of Croatia from 2014-2020 most of the objectives, and the priority measures can contribute to the development for business connections in rural areas, especially through a new form of organization and measures: establishment of producer groups in order to co-finance groups of at least five agricultural producers. This new measure will simplify the procedural, administrative and legal regulations.

Cooperatives in Croatia have a relatively low efficiency, but it is positive that efficiency coefficient is more than one because the primary aim of cooperatives business is not just making a profit. The impact of cooperatives on the economy is more important and has a multi perspective views than just making profit ([www.zadruga.hr](http://www.zadruga.hr)).

Descriptive statistics indicators of cooperatives employment in the Republic of Croatia in 2009 year shows mode (3) median (3) mean (3) minimum value (1) and maximum value (10) (<http://www.coopseurope.coop>). Based on the calculated arithmetic mean and the median, the largest number of counties in the Republic of Croatia has hired three employees at the cooperative, which confirms the calculation mode and shows the most variable in sequence.

Establishing cooperatives should contribute to the generation of new jobs and improving employment in the overall Croatian economy. Based on the available data on employment in the cooperatives in 2007, 2008 and 2009, it is possible to draw conclusions about the impact of cooperative action on employment. In the Republic of Croatia in 2009 were 1 125 cooperatives that had more than 23000 members and 3565 employees. Although the analyzed period had no significant changes in the number of employees of cooperatives in the Republic of Croatia and the general trend was the reduction of the employees' number. Since 2007 until 2009, the number of cooperation employees decreased by 6.60% in average per year ([www.hsz.hr](http://www.hsz.hr)).

Although the respondents think that they have enough knowledge and enough information regarding support for cooperative system, 87% of them believe that there should be more education about cooperatives in current regulatory as well as in the future cooperatives development particularly in the Republic of Croatia accession to the European Union.

National system increasingly recognizes cooperatives as a possible wheel of new employment, creating new income and achieving a significant market share of agricultural food products, but multiple issues are reflected not only as the problems of the past but also by the new cooperative conditions, or the problems of lack information and knowledge of farmers.

### **Conclusions**

The emergence of the first cooperative goes back over two hundred years into the past, when rural entrepreneurs and farmers decided to combine their resources and help each other to overcome common problems, primarily limited market access. The original idea of cooperatives since then didn't change a lot. According to the performed survey research statement, "*I believe that cooperatives can be linked with each other's and achieve a better relationship with the market,*" ranks with the most significant average score of 4.2, therefore it can be concluded that the production organization and distribution of goods are still the most important goal of the cooperative association.

Although national system more and more recognizes cooperatives as a possible new employment engine, creating new income and achieving a significant market share of agricultural food products, multiple issues is reflected not only in the problems of the past but also in the new conditions or the problems as the lack of information and knowledge of farmers. There were some contradictions in the conducted survey results. Although the majority of respondents (62.4%) agree with the statement that the government encourages the cooperatives establishment and cooperative development projects and cooperative systems, in accordance with the economic and social development strategy, and 64.9% of respondents were familiar with measures to encourage cooperatives development in the Republic of Croatia, although 87% of them still believe that they should continue to be informed and have education in the field of cooperatives about current regulatory and future cooperatives development particularly in entering the Republic of Croatia in the European Union, they are still not joining in to the cooperative system. Only 1.7% of the randomly selected respondents is in a cooperative, and 43% of respondents believe that they are more successful as individual farm producers rather than as members of a cooperative.



European countries have specific cooperatives laws, and in some countries, cooperatives are established in accordance with the European Cooperative Society Statute (ECSS). The ECSS was developed in 2003 in order to provide an appropriate legal framework for the cooperatives operation and to facilitate and streamline their cross-border and trans-national activities. The European Commission data show the importance of cooperatives in the European economy because nowadays in the European Union operates approximately 250.000 cooperatives with 163 million cooperative members, or every third citizen of the European Union is in a cooperative. Cooperatives employ nearly 5.4 million people.

In the cooperative organization and cooperative legislation in the Republic of Croatia there are still many understatement and not solved problems. This paper presented and compared the data of the Croatian Cooperatives Association and empirical research. Although cooperatives grew stronger and developed, a negative opinion, farmers ignorance and distrust but also problems in joint ventures and profit sharing are still in effect. Croatian agricultural cooperatives in the future should be compared to the cooperatives in the other European Union environments but with stable even stronger coordination between the state and local communities, and there should be systematic and coordinated approach in making short, medium and long term plans for the cooperatives development and other partnership and some producers groups according to the new rural development plan 2014-2020. By encouraging cooperatives, local governments contribute most to the elimination of the gray economy, unfair competition, and poverty and, most importantly, support employment, growth and development in their communities.

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**ECONOMIC VIABILITY OF SUGAR BEET AND CORN AS ENERGY CROPS  
VERSUS FOOD AND FEED MARKETS: A CASE STUDY IN SPAIN**

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**Abstract**

In the last years many different events, as reforms of the CAP (Common Agricultural Policy), promotion of biofuels due to the international engagements, new energy objectives, etc., have led to a change in the agricultural sector in the EU and particularly in the Mediterranean countries, affecting to a greater extent those regions where agriculture has a higher economic importance, as it's the case of Castile and Leon (Spain). Thus, farmers have been forced to readapt their productive system to the new exigencies of the markets and the policy frame, focusing on new outputs, as energy crops, which compete in both food/feed and energy markets. While technical viability of growing such crops (*e.g.* corn and sugar beet) has been largely proved by different studies and experiences, nevertheless the economic viability of these market orientations versus food/feed markets is not still sufficiently contrasted. This study is an attempt to determine the economic results for these productions, comparing both possible market outputs, using the methodology set by the Economic Accounts for Agriculture (EAA). Results show that both outputs could be options to be taken into account, though an initial support could be needed depending on the evolution of the international context, due to the opportunity cost which implies the energy option versus food/feed markets.

**Keywords:** Energy crops, Sugar beet, Corn, EAA (Economic Accounts for Agriculture).

**Introduction**

Over the last few years, a number of events have produced a deep change in agriculture in the European Union, and particularly in the Mediterranean countries, as it's the case of Spain, affecting more deeply those regions where agriculture has a higher importance from an economic point of view, as it's the case of Castile and Leon region. Thus, the agreements ensuing from the negotiations within the WTO (World Trade Organization), the reform of the CAP and the different CMO (Common Markets Organizations) (in particular that of sugar), along with the region's vocation to produce (greatly influenced by geo-climatic factors) have led to a deep and long-lasting crisis of the agricultural sector in Spain, with a subsequent loss of activity and of the relative importance of the sector as regards its weight in the economy as a whole. Within this framework, farmers feel impotent before the situation of international markets and the region's climatic constraints, limiting the type of alternative crops to be introduced and often reducing competitiveness because of production costs higher than in the European regions of competitors<sup>35</sup>. In spite of it, the region can count on the highest crop yields and surface of sugar beet, as well as maintaining a central role in the production of cereals at the national level, especially regarding corn (Robles and Vannini, 2012).

Likewise, it is noteworthy how important role agriculture has as a supplier of raw material in fulfilling the commitment to reduce emissions of carbon dioxide by 20% (below 1990 level)

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<sup>35</sup> The sparse rainfall, which is in addition very unequally distributed by location and by season, determines the need of irrigation to achieve yields which allow a reasonable profit.

at the latest by 2020, and, in particular, to ensure that biofuels reach a weighting of 10% within overall petrol and diesel consumption (Robles and Perkumiene, 2011). The part it is intended it should play in the requirement to slow down the decrease in biodiversity by 2010 is also crucial. The document "The CAP towards 2020" (European Commission, 2010) reinforces the above mentioned aspects and stress that the future CAP should contain a greener and more equitably distributed first pillar and a second pillar focusing more on climate change and the environment. Thus, further efforts in the field of biomass and renewable energy production will be required to meet the EU energy and climate agenda.

From this perspective, energy crops are an opportunity for the future development of agriculture in this region, enabling farmers to continue their activity in the future (Robles, 2012, Robles *et al*, 2013), but different factors of a legal, technical, economic, or socio-cultural nature have a direct or indirect effect on the value chain for biofuels. These can affect the production sector (area cultivated, costs and the price of raw material), manufacturing (costs and price of biofuel), distribution and consumers (quantities used and prices paid), as noted by Robles and Vannini (2008) and Robles *et al* (2012). In any case, the final decision to include or exclude energy crops when considering alternatives for production lies with the entrepreneur (the farmer) and if energy crops are to be grown farmers must perceive some advantage in the financial results of growing them (Robles, 2011; Robles and Vannini, 2012). In this new context, the present paper aims to analyse the economic accounts for the production of the main local crops which could be grown as raw material for biofuel production, more specifically, corn and sugar beet.

### Materials and methods

The methodology implemented to undertake the work being reported upon here is based on the Rapid Rural Appraisal (RRA). For this purpose, first, we have collected information from different secondary sources, mainly legal and policy documents and reports, which has been contrasted with the primary information supplied by a panel of different stakeholders, which has been interviewed, being experts selected as a function of their prestige, experience and representativeness within each of the following areas: government and administration, farmers and their organizations, supporting institutions and firms, researchers and consultants.

The data obtained in this way were used to undertake an analysis of the circumstances and economic viability of corn and sugar beet as energy and food/feed crops, respectively, applying the EAA methodology (table 1).

Table 1. Economic Accounts.

<b>Production account</b>	<b>Generation of income account</b>	<b>Generation of current profit</b>
Crop Output (Producer Price*yield)	Net value added	Net operating surplus/net mixed income
- Intermediate Consumption	- Compensation of employees	- Non salaried labour
- Consumption of fixed capital	- Other Taxes on production	- Opportunity cost of the own capital
	+ subsidies on production	= Current Profit after distribution
= Net value added	= Net operating surplus/net mixed income	
- Other Taxes on production		
+ subsidies on production		
= Net Value added at factor cost/factor income		

In respect of this, the situation investigated corresponds to the parameters for prices for inputs and end products relating up to the 2011 harvest, using the price trends that emerge from market developments up to that point. For the case of sugar beet, three different situations have been considered, as follows: quota sugar beet (this crop has the institutional support of the CAP and the local administration), sugar beet without quota (it doesn't perceive any subsidy and could be devoted to the food or energy market). As well different levels of yields according to the productivity of the farmers interviewed have been taken into consideration. Moreover the possibility of growing new much more productive varieties specifically focused on energy markets has been considered. Moreover other indicators are calculated:

*Employment Rate*: It represents the labour required by the crop cultivation. It is measured in two different units: Agricultural working unit (AWU)/ha and ha/AWU.

*Break-Event Point (BEP)*: It's the point at which cost or expenses and revenue are equal; there is no net loss or gain.

*Ratio subsidies on product/Crop output*: It represents the importance (in percentage) of the subsidy linked to the energy crop over the total crop output.

## **Results and discussion**

### **Corn**

The economic results, supported by the important rise of prices of the final product, have been, in general, positive for farmers addressing their products to the food/feed market. The main explanatory factor for this trend is the positive evolution of the output prices, set at the maximum levels of the analyzed series (2001-2011), yet the parallel increase of the input prices has hold the benefits rise back. With prices around €0.23 per kilogram for corn, even though an increased cost for inputs has to be taken in consideration, both net income as current profit reach positive values (see table 2). This implies adequate remuneration for the factors land, family labour and capital, together with profits for the entrepreneur that range from about €75.24 for energy market and €1001.24 for food/feed markets. Its inclusion among alternative choices, whether seen from a technical and environmental viewpoint or from an entrepreneurial angle, then becomes feasible. BEP for food/feed market drop relative to the energy market, reaching figures of about 7.74 t/ha versus 10.48 t/ha for energy markets, nevertheless, both values are perfectly achievable in irrigation production systems in the region, with yields over 12 t/ha in normal climatic conditions. As also evidenced by some other studies (*e.g.*, Ericsson *et al*, 2009), the adoption of an energy crop means an opportunity cost which reaches the maximum level for the case of corn (with a profit difference of about 726 €/ha). This amount being for guidance when setting a possible support for energy crops versus food/feed. It must be considered anyway, that there are some other factors apart from the profitability, which could also influence the farmer decision, as diversification of market outputs and minimization of risk. This way, being prices for energy crops subject to a lower prices fluctuation versus food/feed crops, farmers could perceive a comparative advantage when addressing part of the production to the energy markets, which could drive them to grow a certain percentage of the total agricultural area for this productive orientation (Gómez-Ramos, 2009).

### **Sugar-beet**

This crop shows good economic results in any studied scenario, remunerating appropriately every productive factor and providing entrepreneurial profit, too, being this last one considerably higher for the case of sugar-beet with quota (due to the CAP support), reaching values of 1244-2372 €/ha (depending on the considered yields) with respect to 38-776 €/ha for sugar-beet without quota (which could be the one addressed to the energy market). Nevertheless, economic results could improve as a consequence of the prices rise in the international markets, as it's foreseen by the EU (European Commission, 2012), as well as a

result of implementing the farming of new sugar-beet varieties with higher yields (as fuel sugar-beet), currently under experimentation (Merino, 2008) and the reduction of costs due to a possible lesser use of inputs (Salazar-Ordoñez *et al.*, 2013). In such situation, current profit could reach values of 1700 €/ha. This crop has a better economic result (compared to corn), with BEP for energy markets being about 86 t/ha (see table 2). Moreover it should be considered that more efficient farms could reach yields over 150 t/ha, and that the current experimental fuel varieties with higher yields could become a reality in a near future.

Table 2. Economic accounts results.

	Sugar beet without quota		Sugar beet with quota		Fuel variety Sugar-beet	Corn (food/feed)	Corn (energy)
<b>Output</b>							
<b>Yield (t/ha)</b>	115.20	87.10	115.20	87.10	150.00	12.1	12.1
<b>Producer Price (€/t)</b>	26.29	26.29	26.29	26.29	26.29	230	170
<b>Subsidies</b>							
(€/t)			13.85	13.85			
<b>Production Account (€/ha)</b>							
Crop Output (Producer Price*yield)	3028.61	2289.86	3028.61	2289.86	3943.50	2783.00	2057
Intermediate Consumptions	1441.28	1441.28	1441.28	1441.28	1441.28	975.26	975
Fixed Capital Consumption	163.42	163.42	163.42	163.42	163.42	42.75	42.75
Net Value Added	1423.91	685.17	1423.91	685.17	2338.81	1764.99	1038.99
Taxes	103.95	103.95	103.95	103.95	103.95	103.95	103.95
Subsidies on products	0.00	0.00	1595.52	1206.34	0.00	0.00	0
Net Value added at factor cost/factor income	1319.96	581.22	2915.48	1787.55	2234.86	1661.04	935.04
<b>Generation of Income Account (€/ha)</b>							
Net Value Added	1423.91	685.17	1423.91	685.17	2338.81	1764.99	1038.99
Compensations of Employees	30.00	30.00	30.00	30.00	30.00	30.00	30
Taxes	103.95	103.95	103.95	103.95	103.95	103.95	103.95
Subsidies on products	0.00	0.00	1595.52	1206.34	0.00	0.00	0
Net operating surplus/net mixed income	1289.96	551.22	2885.48	1757.55	2204.86	1631.04	905.04
<b>Generation of Current Profit (€/ha)</b>							
Net operating surplus	1289.96	551.22	2885.48	1757.55	2204.86	1631.04	905.04
Non-salaried Labor	92.46	92.46	92.46	92.46	92.46	220.10	220.10
Opportunity cost of the own capital (land and investments)	420.69	420.69	420.69	420.69	420.69	409.70	409.70
Current Profit after distribution	776.81	38.06	2372.33	1244.40	1691.70	1001.24	275.24
Current Profit after deducing just non-salaried labor	1197.50	458.76	2793.02	1665.09	2112.40	1410.94	684.94
Current Profit after deducing just the opportunity cost of the own capital	869.27	130.52	2464.79	1336.86	1784.16	1221.34	495.34
Employment Rate (AWU/ha)	0.008	0.008	0.008	0.008	0.008	0.020	0.020
Employment Rate (ha/AWU)	118	118	118	118	118	50	50
BEP (€/t)	19.55	25.85	19.43	25.69	15.01	0.15	0.15
BEP (t/ha)	86	86	56	56	86	7.74	10.48
Ratio subsidies on product/Crop Output (%)	0.00	0.00	34.50	34.50	0.00	0.00	0.00

Otherwise, sugar-beet for energy use could be an output for the 50% of the surface which is actually being object of deregulation as a consequence of the last reform of the sugar CMO, and for which, it is difficult finding any other suitable crop, due to the climatic and market conditions. The difference between the profit obtained in the two studied scenarios (sugar-beet without and with quota) varies from 1206 €/t to 1596 €/t for yields from 87-115 t/ha, respectively. This will also be the opportunity cost for energy crop (assuming that just sugar-beet without quota would be devoted to the energy market). Otherwise, more productive fuel varieties have a lower opportunity cost of about 681 €/ha vs. quota sugar-beet grown in more efficient farms, and becoming null for those farms with yields under 98t/ha. This cost would disappear as soon as the present quota system does it (foreseen in 2017), later on there will be no opportunity cost for this crop.

### **Conclusions**

Except in the case of sugar beet, once the current quota system will be deregulated, the choice of corn or sugar beet as energy crop means an opportunity cost for the farmer due to the lower profit when comparing with the corresponding food/feed market. Nevertheless, both options could be profitable for the crops subject of this research (though an initial support is advisable in a former stage when introducing these new market orientations).

This factor together with the expected evolution of the demand of these crops up to 2020 according the EC prospects, pointing to a stabilization of the demand in the food/feed markets and an increase in these crops demand for energy use, lead to an expected expansion in the surface devoted to these crops in the region.

The above mentioned facts, together with the expected rising in prices (driven by the increasing demand for energy crops) and the more stable price of irrigation crops (as it's the case of corn and sugar beet), make it advisable to consider the energy option as a part of the productive alternative.

There is an opportunity for these new market orientations, even when growing new surfaces requires the need of investment (as the opportunity cost of the capital has been considered when assessing the costs in this study), yet, in this case, it should be first checked the need of surface in order to get the BEP.

Finally, the current economic situation in the EU, and in Spain in particular, questions the achievement of the foreseen energy crops expansion, due to different factors, as the reduction in institutional funds for research, supporting policies, the cut in energetic expense, or consumer willingness to pay for commodities (outweighing price over environmental or ethic factors).

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**CONSTRAINTS ANALYSIS OF FAMILY AGRICULTURE IN KIRUNDO  
PROVINCE NORTHERN OF BURUNDI**

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**Abstract**

In Burundi, family agriculture occupies more than 90% of the active population. That sector accounts more than 50% of GDP. Before the civil war of 1993, Kirundo province was deemed "breadbasket of the country" because the family farming was market-oriented. Today, this region is the first province in Burundi who accuses a high rate of householders who live in food insecurity. In order to conduct this study, 355 randomly selected farmers were surveyed in all municipalities of the province. This preliminary study revealed that the farmers had as major constraints: the small and land conflicts. The study case had identified 73 farmers among them who had not farmland and/ or land conflicts as constraints of agricultural productivity. Two groups emerged: 42 farmers who were not able to fully exploit their land and another group of 32 farmers with a high agricultural productivity. Results from this study show that the major problems in the first group were: lack of applying soil protection techniques, illiteracy, lack of credit which leads farmers to contract moneylenders, lack of improved seed, etc. Moreover, the main strategy used by the second group to boost their income is the practice of non-agricultural activities which influences whole production system.

**Key words:** Family agriculture, rural economy, pluriactivity, Kirundo, Burundi

**Introduction**

Cycles food shortages facing Africa have forced governments and donors to seek more effective strategies for rural and agricultural development. In Burundi, food prices have doubled or even tripled. For example, the price of rice increased from 600 BIF in 2005 to 1700 BIF in 2012; the price of beans was from 380 BIF in 2005 to 1200 BIF in 2012 (Paridaens et al., 2012). Increasing imports of foodstuffs to bridge the growing gap between demand and domestic production can not continue for a long term. Mellor and Johnston (1984) say that there is a broad consensus on the advantages of agricultural strategies that lead to progressive modernization of small farms in economies where 50 to 80% of the total populations depend on family agriculture. Agriculture and livestock play a vital role in any strategy to reduce poverty. Indeed, 90% of Burundi's population and 69% of the poor live and work in rural areas. In most areas, food production is insufficient to ensure the food security of rural populations and malnutrition rates are particularly high (IMF, 2010). In a very unstable as the prevailing climate in Burundi, the diversity of cultures is the guarantee of a minimum production to sustain the family (MINAGRIE, 2011).

Kirundo province before the civil war of 1993 was "the granary of the country" for the simple reason that region fed many parts of Burundi in particular food legumes (beans and peanuts) and grains (sorghum and maize), therefore a family farm was market-oriented and the number of people who live under poverty line was 28 % (UNDP, 1997). Moreover, food production of these crops are declined to 53,9% from 1996 to 2009 (ISTEEBU, 2010) and

83 % of people live under poverty line (IMF, 2010).



The objective of this study is to analyze main constraints of family farming and propose the main strategy for boosting rural economy.

### **Materials and Methodology**

#### **Area description**

Kirundo is located in Northern Burundi. It is bordered at the North and West by the Republic of Rwanda and Ngozi Province, in the South and East by Muyinga province. Its area is 1,703.34 km<sup>2</sup>. This province accounts 626,355 inhabitants (MININTER, 2011) with population density equivalent to 368 inhabitants/ km<sup>2</sup> (figure 1). In terms of topography, the province has altitude which is between 1,500 and 2,000 m. and is characterized by temperatures between 14.8 and 27.1 ° C and average annual rainfall is between 800 and 1200 mm (URAM, 2007). The rural economy is based on the family farming oriented to the consumption (bananas, sweet potatoes, beans, sorghum, maize, cassava, etc) and livestock which is also traditional with local races such as: cows, goats, pigs, chickens, rabbits, etc.

Figure 1. Demographic density in Kirundo province (UNDP, 2006)

#### **Methodology**

To conduct this study, a first survey was carried out among 355 farmers who were randomly selected in seven communes of the province of Kirundo (Bugabira, Busoni, Bwambarangwe, Gitobe, Kirundo, Ntega and Vumbi) in 2010. The second survey was concerning the case study which has identified 73 farmers among the first survey (355 farmers) who had not a small farmland and land conflict as constraints of agricultural productivity. Two groups emerged: group of 42 farmers exploiting partially their land and another group of 32 farmers who gets a high agricultural productivity. Those farmers were followed during one year from 2011 to 2012. This second survey was completed by analysis secondary data from reports of the Government, Non Government Organisations, etc. To analyze our data, SPSS 16.0 and MINITAB 16.0 were used.

### Results and discussion

The results from the first study show that 54% of farmers have small land (<0.5 ha), 25% of farmers possess an area between 0.5-0.99 ha; therefore, we observe 21 % of farmers who possess than 1 ha of the land (tableau 1). The small farmland is a major constraint to increase agricultural production in that province of Kirundo. These results corroborate with those carried out by the IMF (2009) and (2010) which show that the depletion of land is a major handicap for the development of family farming in Burundi.

**Table 1. Size of farmland in Kirundo province (ha)**

Interval	Average of farmland in hectare by household (ha)	Number of farmers	Percent (%)
< 0,49 ha	0,235	191	54
0,5 -0,99 ha	0,678	89	25
1-2 ha	1,18	58	16
2,01- 5 ha	3,135	13	4
> 5 ha	10,48	4	1
Total/average	0,74	355	100

Source : Our Survey 2010

During the period from 2009 to 2012, the results show that land disputes are respectively 32% and 34% of pending cases (civil and criminal cases) the in court of residence of communes and the High Court of Kirundo. Considering only civil cases, this percentage rises up to 41% in the courts of residence and 70% in the high court (table 2). The land situation is currently in crisis due to the multiple consequences, including crisis characterized by the failure of the current system of land management. This crisis can be summarized in main points, namely: (i) the land issue is a central issue, especially since agriculture now accounts for over 90% of economic activity, (ii) land pressure is growing, leading to the fragmentation of cultivable land and exacerbating land disputes, and (iii) the procedures for registration and changes of land ownership before the Land Code 2011 were inadequate and could not cover the entire national territory (MEATT, 2008).

**Table 2. Land conflicts in Kirundo province**

Type of cases recorded from 2009 to 2010	Case registered from 2009 to 2012 on residence tribunal		Case registered from 2009 to 2012 on residence tribunal	
	Number (n)	(%)	Number (n)	(%)
Conflict cases	2 599	32	1 359	34
Familial cases	1 707	21	256	6
Other type of civil cases	2 008	25	340	8
Criminal cases	1 894	23	2 075	52
Total cases (criminal and civil cases)	8 208	100	4 030	100

Source: Our calculation from residence tribunal and high court data from 2009 to 2012

The impact of land conflicts is numerous: we note that 7 772.5 tons of bananas per year (22% of total banana production at provincial level) are lost because of land disputes. The banana is the main crop which provides much income to rural households. Farmers lose also a significant production estimated at 2.573,3 tones (17 % of total coffee cherries

production at Kirundo province level), main industrial crop for export which permits to farmers to get a lot of income to households and also currency for country. Sorghum provides significant income for farmers in the region. It can be used for the production of beer also for food consumption. That's why to lose 1168.9 tons of sorghum per year (6% of the total sorghum production at the provincial level) is enormous for poor farmers. The main crop in Kirundo region is bean which plays a main role for food and nutrition diet, and procures a lot of income to rural households. However, we observe 7 729.2 tons per year at the provincial level (25% of total bean production at provincial level) lost because of land disputes. Farmers lose also 667.7 tons of cassava per year (31% of total cassava production at the provincial level).

The results from the case study show that the level of education is higher among farmers with high agricultural productivity (6% only of head households are illiterate) than a group of farmers with exploiting partially their land (41% of head households are illiterate). It appears from these results that education level influences significantly the production system, and whole rural economy. Indeed, Lau et al. (1991) found that increasing 10% of education level generated 0.3% of additional economic growth in Sub-Saharan African countries, 1.7% in Latin America and 1.3% in East of Asia, Also the studies carried out by Orivel (2001) confirmed those findings, and showed that farmers who completed four years in primary school had 7.4% higher productivity than their counterparts who do not have attended primary school. Therefore, the increasing of human development capital can improve the technical and managerial practices of farmers and offered to them their chances of success (Gurgand, 2003; Barrett et al, 2001.).

Table 3. Main socio-economic parameters considered

Variables	Farmers with high agricultural productivity	Farmer with partial land exploited
Illiteracy rate (%)	6	41
Annual man-days per household	221	94
Users of mineral fertilizers	25	5
Tropical Animal Unit/ Household	1,38	0,98
Members of local associations (%)	58	32
Cereal-equivalent by active worker (kg)	728,78	304,84
Households with coffee fields (%)	44	19
Households who resort to moneylenders for credit	0	28
Farm income (thousands in BIF) by active work farm	621	234
Investment rate	14	9

Source: Our survey 2011-2012

The agricultural labor in the group of farmers exploiting partially their land is insufficient and is essentially family, the number of farm assets is low, reaching only 2.22 Agricultural assets per household. The number of man-days used annually is 94 man-days per household (71 man-days from family and 23 man-days employees), which gives an average of 8 man-days per month. With this number of man-days per month, it is almost impossible to plow large areas exceed 0.5 ha, that explains many fields abandoned by farmers without being plowed in that group, and therefore the low agricultural production observed in that group. Moreover, the group of farmers with high productivity uses a higher agricultural labor

work (235 man-days annually with 115 man-days employees and 120 man-days provided by family) which allows to these farmers to plow large areas and to achieve other activities related to environmental protection, non-agricultural activities, fertilization, integrated pests management, etc. The impact of that parameter is to increase labor quality, and therefore the agricultural production.

In the group of farmers exploiting partially their land, 5 % only of farmers fertilize their field lands because few farmers possess domestic animal (0.98 Tropical Animal Unit by household) to get organic fertilizer and other are poor to buy mineral fertilizers. Those factors combined with others explain why the low agricultural production obtained by those farmers. Furthermore, the group of farmers with high production gets a higher agricultural productivity because many farmers apply mineral and organic fertilizers in their cultivation lands because they have many domestic animals (1.38 Tropical Livestock Unit by household); they adhere to the local associations in which they follow much training in agriculture, livestock and finance sectors. We observe also 28% of households among farmers exploiting partially their land resort to moneylenders for getting agricultural credit. These moneylenders ask to them a higher interest rate for repayment. Contrariwise, none of member in the group of farmers with high agricultural productivity resorts to moneylenders because their local associations endorse them for accessing to the agricultural credit. Many of farmers among the group with high productivity have coffee fields (44 %) which permit to them getting much income while 19 % only of farmers exploiting partially their lands possess coffee plantations. The group of farmers exploiting partially their land consecrates low percent for investment 9 % while other group allocates 14 % of family income. So, the group of farmers with high productivity gets 728,78 kg of cereal-equivalent and 621 thousands Burundi francs (BIF) by active work farm which are very higher than the agricultural productivity obtained by farmers exploiting partially their land with 304, 84 kg cereal-equivalent and 304 thousands Burundi francs by active work farm. The explanation for that difference within two groups of farmers in production system is given above.

Figure 2. Influence of non farm income on production system (Our survey, 2012)

Typological analysis permits to get three sub-groups for each category of farmers: farmers exploiting partially their lands: 56, 32 and 12 % are classified respectively in A, B, and C sub-groups; farmers with high productivity: 28, 50 and 22 % are also classified in sub-groups A, B and C. The analysis of data shows that non-farm income influences significantly the agricultural production (figure 2).

### **Conclusion**

The main constraints of farmers who exploit partially their land to spur family economy are insufficient of workforce, illiteracy, insufficient animals, etc., in other side, the group of farmers with high productivity has non-farm activities which procures to them much revenue, this non farm income is used to pay salaries, to get organic and mineral fertilizers, improved seeds, etc., and enhances rural economy.

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## STANDARDIZATION OF PRIMARY AGRICULTURAL PRODUCTION AS A CONDITION OF EU INTEGRATION

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### Summary

The awareness of the importance of the healthy products are awoken and production will not distort the natural balance, respectively it will not endanger wildlife and pollute nature. Health care in modern world has become the priority. The risk of disease caused by problems in food production, the application of new technologies, changed lifestyles and dwindling human resistance, increasing daily.

For these reasons, there is imposed idea of a certain international standards, applying to all producers, which would ensure desirable characteristics of products and services such as quality, environmental concerns, safety, reliability, efficiency and the possibility of exchange, all at economic price.

Standards exist for many years, and their value has been confirmed through a global market leadership in agricultural countries. Guided by the example of these countries, agri – food industry of Bosnia and Herzegovina will be able to successfully develop and characterize global competitiveness.

**Key words:** standardization, primary agriculture production, Global G.A.P.

### Introduction

Increasing competitiveness of agriculture is one of the goals of agricultural policy. It tends to create a vital households with intensive production for the market, which will be the basis of long-term and stable agriculture. Today, in the era of globalization and intensive development, requirements for implementation and compliance of international standards for the free flow of agricultural products, environmental protection, quality and product safety are more represented. For achieving these goals, it is necessary to introduce internationally recognized standards: ISO, HACCP standards and GLOBAL G.AP standard.

Today, in a global agriculture, leading standard "Good agricultural practice" is GLOBAL G.AP Standard has been established as a key guarantee of good agricultural practice in the global market. Its significance lies in the fact that it is present in 80 countries, with 97,361 certified agricultural producers. Standard Eure GAP was published for the first time in 1997 by the European food retailer, Retailer Produce Working Group. In September 2007 EUREP group expanded and changed name in the GLOBAL G.AP. We have tried to explain how the introduction of standards is the priority for the economic development of Bosnia and Herzegovina and present the position regarding integrating into a group of internationally recognized food manufacturers.

### Materials and methods

All collected data were analyzed by comparative analysis. Global G.A.P. standard of

certification covers all segments of agriculture: farming, livestock, fruits, vegetables, fish farming, flowers, aromatic plants and herbs, medicinal plant and tropical culture. For successful certification of standards, it is necessary to accomplish 199 requirements and 37 recommendations at 17 production and business areas "from field to table".

Additionally, Global G.A.P. places a strong emphasis on areas related to waste disposal, environmental protection, worker protection, the preservation of the world of wildlife and also food safety products. Taking into account all requirements of the standard, agricultural producers, with quality and on time, meet the requirements of EU legislation, such as the Food Act (Regulation(EC)178/2002) and the Nitrates Directive(91/676/EEC).

**Certified household in Bosnia and Herzegovina according to the Global G.A.P. standard**

According to data from the Institute of Standardization in Bosnia and Herzegovina, there works 16 accredited certification bodies, who audited and certified various international standards. Only two certification bodies TUVSUD Management Services and Mirakol International Co (Agro management DK), deal with GLOBAL .AP certification system. Certification bodies approved by the GLOBALG.AP are accredited by the national accreditation body as directed ISO / IEC 65 or EN 45011 for a particular area.

Certification is carried out in a way to check the entire production process from beginning to an end, including control of all inputs and all activities during the production, storage and transportation. There are four options of certification, of which two are most common in primary agricultural production.

- Option 1, the certification of individual manufacturer or companies, who operates without the help of co-operatives, has small production capacity and shall bear the obligations related to the production and placing on the market
- Option 2, certification of group of producers – That is the manufacturers connected with cooperative agreements, they promote and protect its economic and other interest with the help of cooperative

Option 1	18
Option 2	12 ( 10- 50 subcontractor)
Total	30*

\**www.bas.gov.ba. ( lack of updated database)*

**Global G.A.P. Certified households of the Western Balkans**

Bosnia and Herzegovina	537
Serbia/Montenegro	295
Croatia	132
Macedonia	14
Total	978*

\**www.globalgap. org (august 2012)*

**Global G.A.P. certified households of EU**

Italy	19 508
Spain	15 919
Greece	10 952
Holland	5 125
France	3 893
Germany	3 465
Belgium	3 090
Poland	1 770
Hungary	1 162

Austria	847
Cyprus	709
Portugal	548
Denmark	158
Czech	90
Malta	62
Ireland	46
Romania	36
Slovenia	26
Bulgaria	18
Sweden	14
Slovakia	13
Latvia	5
UK	3
Finland	1
<b>TOTAL:</b>	<b>67 460*</b>

\*[www.globalgap.org](http://www.globalgap.org) (august 2012)

#### Land use in the Western Balkans in 2008 (000 ha)

	BiH	HRV	RS	CG	MK	EU 27
<b>Total area (per 000 ha)</b>	<b>51,2</b>	<b>56,6</b>	<b>77,5</b>	<b>13,8</b>	<b>25,7</b>	<b>4,325</b>
<b>Agricultural area</b>	2136	1289	5065	516	1064	172,485
<b>Utilized arable land</b>	549	769	3093	31	287	106,751
<b>Standing crops</b>	100	342	301	16	36	59,545
<b>Pastures</b>	1049	5	1423	452	603	12,517

\*source: Agrypolicy.net

#### The structure of agricultural households (data 2008)

	BIH	HRV	RS	CG	MK	EU 27
<b>Agricultural households (000 km2)</b>	515	1077	779	43	193	13. 633
<b>% households with less than 2 ha</b>	50%	67%	46%	66%	90%	47%
<b>% household with over 10 ha</b>	4%	5%	6%	5 %	1%	20%

\*Source: T.Volk, 2010



## Results and discussion

Global G.A.P. represents a passport of agricultural product to the European market. The European CAP (Common Agricultural Policy) will introduce a number of changes to which the Western Balkan countries will have to adapt after 2013. The innovations in rural development and agricultural policies are necessary.

At the door of the EU, we face fears regarding the CAP: the future of small producers, fear of competition within the EU, difficulties which bring standards in applying the complex procedures and administrative systems in companies.

Experience of certified companies is, that the optimal costs of production through the entire production process of distribution, increased their competitiveness. Savings are achieved by implementing measures such as the analysis of soil fertility, ensuring the welfare of animals, controlled use of chemical inputs, proper product handling and training of all employees in order to avoid wasting of time and money. By improving quality of final product there will create the preconditions for export on demanding markets of EU and of the world.

## Conclusion

Producers in Bosnia and Herzegovina should strive to offer their products to the international food market, to show that they are serious producers who are not afraid of new challenges of demanding international markets. It is necessary to work on the quality and distinctiveness of the product, and in order to managed, it is necessary to incorporate all of the manufacturer, as well as formation of associations and cooperatives engaged in the production and the formation of a specific database.

The question is whether the chain stores wait to introduce standards or import goods with the certificate. If it is not implemented, customers will buy from those who introduce it, because the awareness about the use of healthy food is increased.

In addition to creating a modern agricultural production through careful management of soil, air and water, the manufacturer raises its competitiveness and opens the door of demanding international markets.

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## PREDICTION OF VEGETABLE PRODUCTION IN REPUBLIC OF SRPSKA

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### Abstract

The subject of this paper is the prediction of production parameters area, yield and total production for major vegetables in the Republic of Srpska: potato, paprika and tomato. The basis for the assessment of adequate model for the prediction has being derived from the data on production parameters of these vegetables during the period of 1996 - 2011 year. On the basis of estimated models, prediction values of the observed parameters have being derived for the year 2016.

The prediction is based on modern quantitative methods, specifically the method of time series analysis and the appropriate ARIMA models. The choice of the form of model is the result of qualitative analysis and statistical criteria.

Prediction of the production area shows that there will be changes in the production structure of the observed vegetables in the Republic of Srpska in 2016. year. Areas of potatoes will be reduced by about 600 ha, while the production area for paprika and tomatoes is going to be increased by the same amount. Yields of potatoes and paprika, in the analysed period are characterized by stability, while smaller fluctuations are indicated for yields of tomatoes. Tendencies that characterize the area and yield of crops observed, have a direct influence on their production. Prediction for potato production shows that the total volume of production in 2016 will be lower by about 2,000 tons compared to 2011, what is the result primarily of the reduction of the area under potatoes. Predicted tomato production will increase by about 500 tons.

The results of prediction can be used as a basis for qualitative analysis of the production and development of vegetable growing in the Republic of Srpska, as well as for general policy and strategy planning for vegetable production in the future, and design of agricultural policy measures to encourage the development of production, consumption, processing and export of vegetables observed.

**Keywords:** vegetable production, forecasting, Republic of Srpska

### Introduction

Vegetable production is one of the most intensive types of plant production, which is confirmed by the yields per unit area and realized economic effects. Bearing in mind the economic benefits of this type of agriculture production for the farmers and for agriculture sector in general, the main directions of its future development are optimal use of available production capacity, increase in production volume and changes in production structure.

The subject of this research is the prediction of the production characteristics of major types of vegetables in the Republic of Srpska: area, yield and total production. The analysis included the following vegetables: potatoes, paprika and tomatos. The analysis covered the period from 1996 till 2011. The aim of the research is to show the importance of vegetable production in Republic of Srpska. The results of the forecasting should serve as a basis for

quantitative analysis of vegetable production in the Republic of Srpska. Also, the results of the research could help in defining the concept of agricultural policy measures for stimulating the development of production, consumption, processing and exporting of types of the vegetables observed in the paper.

### Materials and methods

In the market economy, successful production depends on monitoring, analysis and prediction of the results and most important factors that influence these results. Situation analysis and prediction are based on a series of data arranged at equal intervals, and the analysis of time series of observed phenomena. Data used in this paper are related to the production results such as area, yield and total production of potatoes, paprika and tomatoes in the Republic of srpska in the period from 1996 till 2011.

The paper objective to forecast production upon the analysis of the observed time series. This process is managed by the data available from the past on the basis of which time-series models are formulated and evaluated, which were then used to predict future values of series. The verification of the rated models is performed and for this purpose statistical tests and criteria were used which verify the validity of assessed models.

The analysis and prediction method applied in this paper was based on the class autoregressive model of mobile environments (ARIMA (p, q)). The assumption of this models is that the current value (member) series depends on the values of the previous members of the series, the current values of the random process and the previous values of the random process of white noise. For the time series in which the effect of trend, cyclical and seasonal components can be observed, the application of these models includes prior removal of their influence. To eliminate the influence of systematic components of the time series, the operator of differentiation is used. Using the difference of the first order removes the linear trend, other differences removes the quadratic trend, and k - differences eliminates the influence of trend polynomial k - th degree. Trough the differentiation process, we get the class of ARIMA (p, d, q) model, where the values of the original series replace certain differences. Class ARIMA model can be used for analysis and modeling of a number of stationary and non-stationary processes.

### Research results and discussion

#### Analysis of the situation

Since the beginning of the analysed period (1996), the area under potatoes in the Republic of Srpska shows the trend of constant decrease (-0.33%). In average, during the analyzed period, this vegetable crop was represented by an area of about 17,400 ha (Table 1). The volume of production, as opposed to the production area, is characterized by the tendency of increase (1.87%) but, at the same time, the production volume shows a large oscillations (Cv = 14.72%).

**Table 1 Basic indicators of potato production in the Republic of Srpska, 1996-2011**

Production parameters	Average value	Variation interval		Coefficient of variation (%)	Rate changes (%)
		Minimum	Maximum		
Harvested area (h )	17,370	14,539	21,408	12.51	-0.33
Production (t)	169,304	122,933	208,447	14.72	1.87
Yield (t/h )	9.9	6.4	12.3	18.64	2.19

The average yield of potatoe had a tendency of increase in the analyzed period, by the average rate of 2.19% per annum, as well as the variability, expressed by the value of the coefficient of variation of 18.64%.

The forecast model (Table 2) shows that the potato production area in the current period is under a significant influence of the size of this area from the previous period. Predicted values (Table 3) confirm the perceived tendency of decrease. Between 2012 and 2016, the area under potato will be constantly reduced to a level of about 14,500 hectares.

**Table 2. The parameters of the model for prediction of the potato production area**

CaseNo.	Forecasts; Model:(1,0,0) Seasonal lag: 12 (rspovrce) Input: POVKRO Start of origin: 1 End of origin: 16		
	Forecast	Lower 50,0000%	Upper 50,0000%
17	14685,15	2623,100	82213,3
18	14634,50	1281,288	167151,1
19	14584,04	739,027	287803,3
20	14533,78	464,578	454671,7
21	14483,70	308,576	679824,2

**Table 3. Prediction of potato area (2012-2016)**

Model for the analysis and prediction of potato production in the Republic of Srpska (Table 4) shows that the production volume of the current year is significantly influenced by the production volume from the previous year. Tendency of the increase in potato production in the analyzed period will unfortunately not be characteristic of the period of predictions (Table 5). Predicted values indicate that the potato production will decline within forecast period, and at the end of the prediction period will be at the level of about 172,700 tons.

**Table 4. The parameters of the model for prediction of the production volume**

CaseNo.	Forecasts; Model:(1,0,0) Seasonal lag: 12 (rspovrce) Input: PROIZKRO Start of origin: 1 End of origin: 16		
	Forecast	Lower 50,0000%	Upper 50,0000%
17	174557,4	21154,81	1440348
18	174083,2	8805,11	3441747
19	173610,4	4492,19	6709548
20	173139,0	2546,57	11771556
21	172669,0	1544,25	19306901

**Table 5. Prediction of potato production volume (2012-2016)**

The forecast model for prediction of the potato yield, (Table 6) shows that the yield for the curent year is significantly conditioned by the yield of this crop in the previous year. Expected values for the period 2012-2016 year (Table 7) show that the yield of potato will be reduced. The average yield by the end of the forecast period will be at the level of about 10 tons per hectare.

**Table 6. The parameters of the model for prediction of the potato yield**

CaseNo.	Forecasts; Model:(1,0,0) Seasonal lag: 12 (Republika srpska povrce) Input: PRINKROM Start of origin: 1 End of origin: 16			
	Forecast	Lower 90,0000%	Upper 90,0000%	Std.Err.
17	10.95001	8.070526	13.82950	1.634855
18	10.43843	7.167955	13.70890	1.856841
19	10.16293	6.787530	13.53833	1.916413
20	10.01457	6.609344	13.41979	1.933346
21	9.93467	6.520849	13.34849	1.938229

**Table 7. Prediction of potato yield (2012-2016)**

Paprika is, in the analyzed period, grown on average 2,383 hectares, with a decreasing trend of the total production area (Table 8). The volume of production and yield of paprika are showing a significant variability in the analyzed period, but they also have significant increasing trend.

**Table 8. Basic indicators of paprika production in Republic of Srpska, from 1996 to 2011**

Production parameters	Average value	Interval of variation		Coefficient of variation (%)	changes rate (%)
		Minimum	Maximum		
Harvested area (h )	2,383	2,079	2,602	6.41	-0.91
Total production (t)	22,446	13,071	28,806	20.62	3.19
Yield (t/ha)	9.5	5.8	12.2	22.10	4.15

Model for predicting the total production area for paprika, assessed on the basis of the changing values for production area in the analyzed period (Table 9), shows that the share of paprika in production structure is significantly affected by the share of this crop in the previous period

**Table 9. The parameters of the model for prediction of the paprika production area**
**Table 10. Prediction of production area for paprika (2012-2016)**

Forecasts; Model:(1,0,0) Seasonal lag: 12 (rspvrce) Input: POV/PAP Start of origin: 1 End of origin: 16				
CaseNo.	Forecast	Lower 50,0000%	Upper 50,0000%	Std.Err.
17	2150,106	2066,377	2233,835	120,9227
18	2202,857	2098,603	2307,111	150,5653
19	2241,991	2128,007	2355,975	164,6178
20	2271,023	2152,023	2390,023	171,8623
21	2292,561	2170,888	2414,234	175,7220

Predicted values of the production area for paprika by 2016. are characterized by a slight increase to a level of almost 2,300 hectares (Table 10). Although we can notice that, by the end of the forecast period, there is a tendency of a slight increase in size of production area, it will not reach even average, nor the maximum of the recorded area in the analyzed period.

**Table 11. The parameters of the model for prediction of the production volume for paprika**
**Table 12. Prediction of the production volume for paprika (2012-2016)**

Forecasts; Model:(2,0,0) Seasonal lag: 12 (rspvrce) Input: PROIZPAP Start of origin: 1 End of origin: 16			
CaseNo.	Forecast	Lower 50,0000%	Upper 50,0000%
17	24031,27	4015,299	143825
18	23968,43	1824,170	314930
19	23906,60	1000,377	571310
20	23844,98	604,056	941275
21	23783,54	387,669	1459122

The volume of production shows a significant increase throughout the analyzed time period and this at a rate of 4.15% per annum. The forecast model (Table 12) shows that the level of production volume for paprika in a current year is significantly influenced by the production from the previous two years.

Production of paprika in the future period has a tendency to decrease, what is indicated by the predicted value at the end of 2016 (Table 12). It is expected that the production of paprika at that time will be at a level of almost 23,800 tons.

The yield for paprika in the analyzed period shows some oscillations, and in some years there has been sensible decline in yields, but significant increase already in the next year. The forecast model for predicting the yield for paprika in the future (Table 13) indicates that the yield of the current year is under a significant impact of production results from the previous period.

**Table 13. The parameters of the model for prediction of the yield for paprika**

Forecasts; Model:(1,0,0) Seasonal lag: 12 (rspovrce)			
Input: PRINPAP			
Start of origin: 1 End of origin: 16			
CaseNo.	Forecast	Lower 50,0000%	Upper 50,0000%
17	11,59296	8,010574	16,77742
18	11,58593	6,869643	19,54013
19	11,57890	6,105044	21,96069
20	11,57188	5,526646	24,22960
21	11,56487	5,062449	26,41926

**Table 14. Prediction of the paprika yield (2012-2016)**

Anticipated yield values for the period 2012-2016 (Table 14) show that the yield for paprika is expected to decline by about 100 kg per hectare each year and will reach the level of about 11.6 tonnes per hectare.

Basic indicators for tomato production in the Republic of Srpska in the analyzed period are given in Table 15.

**Table 15. Basic indicators for tomato production in the Republic of Srpska, 1996-2011**

Production parameters	Average value	Interval of variation		Coefficient of variation (%)	changes rate (%)
		Minimum	Maximum		
Harvested area (h )	2,094	1,851	2,439	7.77	-0.25
Total production (t)	19,502	12,012	26,620	21.85	1.25
Yield (t/ha)	9.4	5.6	13.6	23.09	1.48

In a period from 1996 to 2011, tomato has grown on an average area of 2,100 hectares. During this period, the production area was relatively stable (CV = 7.77%), and from year to year showed a tendency to decrease at a rate of - 0.25%. The volume of tomato production in this period showed significant variability with a tendency of increase at a rate of 1.25% per annum and the average value stood at 19,500 tonnes. Tomato yield is characterized by the tendency to increase at a rate of 1.48% per annum.

For the analysis of the production area under tomato, the forecast model was developed (Table 16) that indicates that the production area of the current year is significantly influenced by the production area for tomato in the last year.

**Table 16. The parameters of the model for prediction of the production area for tomato**

**Table 17. Prediction of the production area for tomato (2012-2016)**

Forecasts; Model:(1,0,0) Seasonal lag: 12 (rspovrce)				
Input: POVPAR				
Start of origin: 1 End of origin: 16				
CaseNo.	Forecast	Lower 50,0000%	Upper 50,0000%	Std.Err.
17	1896,766	1803,589	1989,944	134,5684
18	1929,726	1814,900	2044,552	165,8335
19	1953,462	1828,881	2078,044	179,9221
20	1970,557	1841,206	2099,908	186,8111
21	1982,868	1851,111	2114,625	190,2860

Unlike the analyzed period, in the forecast period, it will come to a slight increase in the production area for tomatoes. This is confirmed by the values of production area forecasted for a period 2012-2016 (Table 17). In the last year of the forecast period, tomato will be grown at the area of about 2,000 acres.

The forecast model for predicting tomato production (Table 18) shows that the production of the current year is significantly influenced by the production level of the previous year. The estimated value of the production volume (Table 19) shows a tendency of increase from year

to year within forecast period. The expected production in 2016 will be at a level of about 18,000 tons.

**Table 18. The parameters of the model for prediction of the production volume for tomato**

**Table 19. Prediction of the production volume for tomato (2012-2016)**

CaseNo.	Forecasts; Model:(1,1,0) Seasonal lag: 12 (rspovrce) Input: PROIZPAR Start of origin: 1 End of origin: 16			
	Forecast	Lower 50,0000%	Upper 50,0000%	Std.Err.
17	17529,70	13708,35	21351,05	5507,621
18	18369,20	14331,28	22407,12	5819,762
19	17862,67	12854,78	22870,56	7217,756
20	18242,63	12896,80	23588,45	7704,811
21	18038,75	12098,80	23978,70	8561,114

Model for the analysis and prediction of tomato yield (Table 20) shows that the yield for the current year is significantly depending on the yield achieved in the previous year.

Tomato yield values predicted on the basis of assessed models (Table 21) show that the yield is going to decrease or increase, alternating from one period to another and at the end of 2016 will be at a level of 10 tons per hectare.

**Table 20. The parameters of the model for prediction of the tomato yield**

**Table 21. Prediction of the tomato yield (2012-2016)**

CaseNo.	Forecasts; Model:(1,1,0) Seasonal lag: 12 (rspovrce) Input: PRINPAR Start of origin: 1 End of origin: 16		
	Forecast	Lower 50,0000%	Upper 50,0000%
17	9,45444	7,781367	11,48724
18	9,97038	8,094727	12,28066
19	9,76665	7,560101	12,61722
20	10,01417	7,599739	13,19566
21	9,98172	7,354042	13,54828

## Conclusions

Prediction of vegetable production area indicates that there will be changes in the production structure of the studied crops in the Republic of Srpska by the year 2016. Area under potatoes will be reduced for about 300 ha, while the production area for paprika and tomatoes will increase for the same amount. The yields for potatoes and paprika, are characterized by stability in the period of forecast, while small oscillations are indicated in the case of the yield values for tomato. Tendencies that characterize the production areas and yields of the observed vegetable crops are directly reflected on their production volume. Predicted production volume for potato at the end of 2016 will be lower by about 2,000 tons compared to 2011, and this is primarily caused by reduced production area. The production volume for tomato is predicted to increase by about 500 tonnes at the end of the prediction period.

Predicted results can serve as a basis for quantitative analysis of the vegetable production in the Republic of Srpska, as well as for defining agricultural policy concept and strategy measures for vegetable production in the future to stimulate the development of production, consumption, processing and exporting of the vegetable crops observed in this paper.

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## **INSTRUMENTS OF ECONOMIC MEASURES OF AGRICULTURAL POLICY OF THE REPUBLIC OF SRPSKA**

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### **Summary**

The agricultural sector is the most reputable resource in the Republic of Srpska. Development of the agricultural sector, in addition to natural and human resources, is influenced by the action of certain measures of agricultural policy, which essentially represent a strategy of realization of set goals in terms of integration of the agriculture sector in the development of economy. One of the most important groups of agricultural policy measures are economic, which are primarily related to issues of economic problems of the sector. This paper provides an overview of economic measures and its instruments in the framework of agricultural policy of Republic Srpska for period of time 2007 – 2011. By the describing of economic measures and its instruments were used secondary data sources. The findings of this paper implies that economic measures and its instruments did not contribute to improve the competitiveness of agricultural sector of the Republic Srpska.

**Keywords:** agriculture, economic measures, instruments, Republic of Srpska.

### **Introduction**

The agricultural sector in the economy of the Republic of Srpska entity occupies an important place. The contribution of agriculture to overall economic development of the Republic Srpska is reflected in the share of the sector in gross domestic product (over the period 2007 - 2011 was average 10%), while in the total employment about 30%. In foreign trade, agriculture sector of Republic Srpska recorded continuously deficit, with almost all of major trading partners: CEFTA countries and EU. In Bosnia and Herzegovina, two entities; RS and FBiH and District Brcko, within the same country create their own policy for development of agriculture and rural areas. In 2006, the Ministry of Agriculture of Republic of Srpska defined the Strategy of Agricultural Development for period 2006 – 2015. The Strategy represented basic principles for determining of objectives, measures and instruments of agricultural policy, contained in Law on Agriculture. According to the same law, the strategic goals of agricultural development are being implemented through four main groups of measures: (1) *market – price policy*; (2) *structural policy measures*; (3) *measures of land policy*; (4) *the policy of subsidies*. Generally, one of the most important groups of agricultural policy measures is economic measures. The following sections describe the economic measures and their instruments in the framework of agricultural policy, as well as the their impact on competitiveness of agriculture in Republic Srpska.

### **Materials and methods**

In this paper as secondary sources of data were used: (1) the publication about corporate investments in agriculture sector of the Institute for Statistics of Republik Srpska; (2) report of state of banking sector of Republic of Srpska and overview donor activities in BH; (3) database of loan of Investment Development Bank of Republic Srpska; (4) database on

realised subsidies of Ministry for Agriculture of Republic Srpska; (5) Strategic documents: *Agriculture Development Strategy, Strategic Plan for Rural Development and Strategy for Advisory Services of Republic Srpska* ; (6) publication of Foreign Trade Chamber of BH; (7) data of domestic authors published in certain articles and publication. By the description of certain economic measures such as agricultural investment and agricultural subsidies, on the basis of available data, results are expressed in absolute numbers and relative relations (percentage) (table. 1 and table 2).

## Results and Discussion

### Agricultural investment

Investments are a precondition for development. Development is possible to some extent by increasing the efficiency of utilization of capital funds, but for the more serious and intensive development it is inevitable to invest financial funds for the expansion and replacement of production and other capital funds. The transition process in Republik Srpska, BiH requested more significant investments in order to successfully bridge the transition process. The needs for investments have increased and were caused by significant war damages which (depending on the source) are estimated at 50-100 billion USD. According to a survey conducted in 1998. (Vasko, 2000) it was found that the war damage in agriculture ranged from 45% to 95% for agricultural equipment and 16% to 83% for cattle. For example, in 2009, the donations to the sector of agriculture and forestry were 14.3 million EUR, and in 2011, it was 4.6 million. Loan funds of the World Bank and other creditors in the same sector in 2009 amounted to 32.2 million EUR, and in 2011, 4.8 million EUR (Donor Mapping, 2012). About 9.4 million EUR of these grants and loans to the agricultural sector in Bosnia and Herzegovina in 2011, accounted for only 1.2% of its GVA of its agriculture in that year, and show modest achievements that can be expected from investments provided by external sources. One of the problems that often cannot give a reliable assessment of the level of investment in agriculture in BiH is partial monitoring of this phenomenon by the statistical agencies. Specifically, the statistics is only monitoring the corporate investments, but in the case of Republic of Srpska it means that a share of investments undertaken by farms is not monitored, although they are still a majority of holders of agricultural production in RS. Data on corporate investments in agriculture suggest that investments in the last five years (2007-11) have increased slightly.

Table 1: Financing of gross fixed capital by main sources 2007- 2011.

Indicators	2007	2008	2009	2010	2011
Share of Agriculture, hunting and forestry of total investment in RS (%)	2.1	2.4	3.3	2.9	3.6
Structure of financing of gross fixed capital by main sources (%)					
<b>from own financial assets</b>	<b>58.2</b>	<b>61.3</b>	<b>53.5</b>	<b>64.1</b>	<b>66.9</b>
from joint assets	2.8	2.1	4.0	2.3	0.6
<b>from financial credits</b>	<b>21.8</b>	<b>28.9</b>	<b>30.1</b>	<b>15.8</b>	<b>18.7</b>
financial leasing	13.0	1.1	3.3	3.1	3.3
from assets of budgets and funds	0.4	5.6	1.6	5.8	5.0
from other sources	3.8	1.0	7.4	9.0	5.4

Source: Own calculations of authors based on data by the Institute of Statistics (Gross fixed capital formation, Investment, Statistical Bulletin No. 3/2009, 4/2010, 5/2011, 6/2012.)

Investments in agriculture accounted for a 2.1% to 3.6% of total investment in the RS, which is significantly below the share of this sector in the creation of the country's GDP. In terms of sources of investment in agriculture, own funds are predominant, with average share of 60.8% of total investments in agriculture. Share of loans in financing agriculture varies, but on average it is about 20% of total investments in agriculture. Funds from leasing has never been a significant source of investments in agriculture in the RS. Such a structure of investment indicates that the capital market in the RS is still conservative as it relies on its indigenous funds and loans. Banks mainly provide loans to companies, cooperatives and other legal entities, and to a lesser extent to unregistered farmers that mainly borrow funds from microcredit organisations. Out of total lending of banks in the RS, only 1.84% is in the agriculture, hunting and fishing sectors, which is significantly below than the contributions of this sector to the creation of GDP (Report on the state of the banking sector of RS, 2012). Credit for "*opening the door*" to agricultural credits goes to the development projects financed by international financial and other organizations (World Bank, IFAD, USAID, etc.) and the Investment-Development Bank of the Republic of Srpska. For example, IFAD in all four post-war projects in BiH had components for providing credits to farmers and SMEs within which by the end of 2010, under favourable conditions, approved about 16,000 loans with a total value of 67 million KM (Vasko et al, 2011). Since its inception, the Investment Development Bank of the Republic of Srpska provided 1245 microcredits amounting of 23.7 million KM for agriculture in a total (average loan is in the amount of 19 000 KM) and 91 credits for companies in the amount of 71.1 million KM (average loan is in the amount of 0.78 KM), which makes 10% of its total loans. Generally, the level of investment in agriculture in BiH is modest, and in addition to the lack of own resources limitation, there is the lack of credits and unfavourable lending conditions (high interest rates, short repayment periods, without or with a short grace period).

### **Agricultural insurance**

Agricultural insurance is a risk sharing mechanism between agricultural producers and insurers. Ranges of agricultural insurance in BiH were not significant even earlier, but now in the post-war period, it can be concluded that agricultural insurance does not exist at all. Insurance companies avoid to offer this kind of insurance contracts, justifying it with the high risks, and on the other side agricultural producers avoid to insure properties and production because insurance premiums are considered expensive, and recoveries are insecure and complicated. Despite the fact that the Ministry of Agriculture, Forestry and Water Management, in its subsidy program, has a measure of sub financing the insurance premiums with 50%, the opportunity was only used by 30 farmers in 2011 . On the other side, the damages caused by devastating floods and droughts in that year were estimated at more than a billion KM.

### **Agricultural subsidies**

The Republic of Srpska encourages the development of its agriculture through financial incentives since 2000. In this period, the amount of the subsidy has increased, in both absolute and relative terms compared to its budget, the total GVA of agriculture and total GDP. In the period 2007-11 the average annual subsidy for agriculture in RS amounted to 6.1% of its budget, 9.91% of its agriculture GVA and 0.98% of its GDP (Vasko et al., 2012). Noting that after a continuous progression, before the end of this period there was a decline due to the reduction of the percentage allocations for subsidies due to the outbreak of financial and economic crisis. It is evident that that the increase in GVA of Srpska agriculture during

2000 - 2008, was to a large degree, dependent on the increase in subsidies for agriculture, when the statistical significance was noted in the parameters of regression models and coefficient correlations (Vasko and Ostojic, 2008). Agricultural Development Strategy of the Republic of Srpska (2006), includes the ratios of allocations of subsidy funds to the three main groups (for first implementation Phase of Agricultural Development Strategy (2007 - 2009): 40%: 40%: 20% and for second implementation Phase (2010 - 2015): 30%: 50%: 20%), which in the analysed period have not generally been implemented. The goal to reduce direct payments in favour of increasing subsidies to support investments has not been achieved. Agriculture is still subsidized in the conservative way with the dominance of direct payments that are tied to the amount of produced or sold products, or subsidizing purchases of inputs for agricultural production.

Table 2: The structure of agricultural subsidies in the Republic of Srpska during 2007-11

Year	1. Support to production and income		2. Support to long term investments		3. Support to rural development		4. Interventions	Total
	Mil.	Structure (%)	Mil.	Structure (%)	Mil.	Structure (%)	Mil.	Mil.
<b>2007-09. Goal</b>		<b>40%</b>		<b>40%</b>		<b>20%</b>		<b>100%</b>
2007.	48.0	75.9	8.6	13.6	6.6	10.5	0.9	64.1
2008.	86.7	80.8	12.7	11.8	7.9	7.3	0.5	107.8
2009.	40.5	74.2	5.3	9.7	8.8	16.1	0.9	55.5
<b>2010-15. Goal</b>		<b>30%</b>		<b>50%</b>		<b>20%</b>		
2010.	37.5	47.3	20.5	25.8	21.3	26.9	16.5	95.8
2011.	49.1	62.3	23.3	30.0	5.2	6.8	2.6	80.2

Source: Own calculations of author based on data by the Ministry of Agriculture on paid subsidies 2007 -2011.<sup>36</sup>

General characteristic of agricultural subsidizing policy in the RS is the chronic lack of funds. Subsidies in support of production and income are almost exclusively linked to specific products or production lines, and are paid for achieved production or sale (milk, meat, grain, industrial crops, vegetables, fruits, seeds, seedlings, etc.) and for subsidizing the costs of inputs (diesel fuel, fertilizers). In supporting investments, most of the money goes for purchase of new agricultural equipment, constructing and rehabilitation of buildings for animals, planting perennial plantings and upgrading processing facilities of food industry. Support to the improvement of rural livelihoods and rural infrastructure construction noted significant fluctuations. In 2010, 18 millions KM were allocated for these purposes while in 2011, allocation to this segment was only 1.5 million KM. In late 2009, the RS Parliament adopted a Rural Development Strategic Plan for the period 2009-2015. This plan included measures to support rural development modelled on the second pillar of the CAP, with the intention to make rural development policies in the RS closer to the EU rural development policy. The Plan, as well as Agricultural Development Strategy envisages gradual increase in resources allocated to subsidizing rural development. After two years of validity of the Rural Development Strategic Plan, it is evident that there are major differences, both in terms of the type and scope of the measures implemented and the amount of the subsidies for rural development. For example, in 2011, implemented funds out of agricultural budget for these purposes were twice lower than projected.

### Agricultural taxation

The area of taxation generally characterized by two main groups of taxes: *direct and indirect*. Direct taxation in agriculture refers to the application of income tax on earnings from

<sup>36</sup>Mentioned amounts and percentages are calculated on the basis of obligations for the year (including debts paid for agricultural budget for coming year).

agriculture and forestry activities as well as taxes on property. As in most countries, in the Republic of Srpska also, direct payment of income tax is linked to the cadastral income. By 2012, farmers were exempted from paying of property tax. After the entry of the new Law on Property Tax (*Official Gazette of RS, number 110/08; 118/09*), farmers pay tax if they have agricultural land which is not cultivated, and agricultural buildings, which do not serve in their own agricultural production. Agricultural areas to be treated, as well as property to be used for agricultural production, are exempted from taxation. Indirect tax collection in agriculture is related to the value-added tax (VAT). According to the data of the Indirect Taxation Authority in 2010, about 610 farms in the Republic of Srpska were covered under VAT system, which is about 1.1% of the total number of registered farms (55491), while the number of farms, users fixed fee for the same period was 3180 or 5.7 %. The rest are the farmers, who are out of VAT system and are not users of lump-sum compensation. In terms of effects of indirect taxes on agriculture, it can be also concluded that farmers are substantially burdened by the tax, which generally results in a reduction of their income.

### **Price policy**

One of the most sensitive issues faced by domestic agricultural production is the *price policy* and the protection of domestic production. For domestic agricultural production, it can be said that it has very low production and price competitiveness, while, on the other hand, the developed countries, because of the high price and product competitiveness, make a big pressure in terms of liberalization of trade in agricultural products and the reduction of price subsidies. Establishment, management and implementation of special forms of protection prices are all important factors in price policy, because the development of certain branches of production and agriculture is ensured and harmonized through them, as well as the entire agriculture. So the price is affected by the direction, scope and structure of production, market stability, and size of income and living standards of producers and consumers, and others. Agricultural prices in the domestic market are formed freely, but in order to avoid incompliance between supply and demand, there are the state laws that prescribe and determine the protective prices (regulated prices). According to the Article 7 of the *Law on Agriculture of the Republic of Srpska*, the formation of the three types of administered prices: guaranteed, orientation and import-price threshold are suggested. Therefore, it is about the prices that have agricultural policy character given that they are defined by the Government on the proposal of the relevant Ministry. Mechanisms of orientation prices were applied only in wheat in the past, while other agricultural products applied any market-defined price. The reason for controlling and activating the orientation prices for wheat is its strategic importance for the Republic of Srpska. Prices of agricultural products are one of the factors that determine the position of agriculture as well as agricultural production trends. Based on the purchase and/or sale prices producers often make decisions about the intensification of certain production or in some cases, suspend production. The growth of prices in agriculture has a much bigger impact on the production of certain agricultural products, than the overall growth rate in agriculture. The prices in this sector have a direct impact on the income of both producers and consumers since those are products which are used in the daily food of the population.

## Foreign trade policy

Since the adoption of the Law on Foreign Trade Policy of 1998, Bosnia and Herzegovina is taking the first important steps towards the *liberalization of trade*. CEFTA countries are the most important trading partners of Republic Srpska in both the general trade as well as the trade of agricultural and food products. The agricultural products within CEFTA are permitted for tariff concessions, mostly transferred from bilateral agreements and referred to the full liberalization. According to the CEFTA agreement, Bosnia Herzegovina with nearly all member countries of CEFTA fully liberalized trade with agricultural products (headings of 01-24). One of the main objectives of the foreign policy of Bosnia and Herzegovina is accelerating the process of integration in the EU. For Bosnia and Herzegovina, the implementation of *the Stabilisation and Association Agreement* with the European Union was an important step towards trade liberalization and integration into the global economy. Stabilisation and Association Agreement is an international agreement that enabled Bosnia and Herzegovina to acquire the status with EU. Parallel with the signing of the SAA, Bosnia and Herzegovina also signed the Interim Agreement on trade applicable until the completion of ratification procedures of SAA. Through this agreement, the EU concessions on imports of agricultural products from Bosnia and Herzegovina were reflected in the abolition of quantitative restrictions and measures the same effect on imports of agricultural products originating in Bosnia, except for the following products: live animals beef, sugar, wine from fresh grapes. According to Agreement, Bosnia and Herzegovina concessions on agricultural imports from the EU were reflected through the elimination of all quantitative restrictions and measures having equivalent effect on imports of agricultural products from the EU.

## Conclusions

Despite donations and loans for development, the level of investments in RS is inadequate, and its economic system is still immature as agricultural production ranges between 2.1% and 3.6% of the total investment in the RS and is significantly below the share of this sector in the creation of the country's GDP. Only 1.84% of total lending of banks in the RS is in the agriculture, hunting and fishing sectors, which is significantly below the contributions of this sector to the creation of GDP. Given this situation, agricultural production in RS will remain vulnerable to natural disasters and calamities. Agro -insurance will not be a significant measure of its farm policy for long mostly because of expensive insurance premiums. The funds allocated for the support of agriculture are relatively modest. The modalities of distribution of these funds are generally not harmonized with the Common Agricultural Policy of the EU, although the harmonisation of all policies, including agricultural, with those applied in the EU. In terms of effects of indirect taxes in agriculture, it can be concluded that farmers are substantially burdened by the tax, which generally results in a reduction of their income. Mechanisms of *“specific price protection policies”*, were applied only in wheat in the past, while other agricultural products are exchanged for market-defined price. The liberalization of trade in agricultural products, regardless of the number of advantages that trade agreements can have for BiH and RS standing in the global economy, has generally had a negative impact on agricultural production. In general, these findings (low level of subsidies, tax burden on farmers (VAT), the disorganization of the market of agricultural products, as well as the low level of foreign trade protection) suggest, that economic instruments of agricultural policy measures did not contribute to improve the competitiveness of the agricultural sector of the Republic Srpska.

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**STRATEGIC NEXSUS OF ECOTOURISM AND ECOLOGICAL FARMING IN  
FUNCTION OF SUSTAINABLE DEVELOPMENT IN RURAL AREAS OF  
MONTENEGRO**

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**Abstract**

The demand for eco products grows in every part of life and work. Global ecologic trends are being reflected on tourism as an active component of society. This is how ecotourism became, one of the modern tourism forms that is less destructive than the other. It creates at the same time chance for development of underdeveloped, mostly rural regions. Ecotourism development has a positive effect on complementary branches of economy, agriculture before all. There are multiple positive reflexive ecotourism effects on ecological farming and vice versa. Intensive ecotourism development and its linking to agriculture creates possibility of additional revenue for agricultural households in rural areas which contributes to the quality of life and to suspending of the demographic downfall. Eco agricultural production significantly contributes to the competitiveness of ecotouristic product on one side, while on the other besides creating possibility for producers to directly place their products through ecotourism, also offers the possibility to valorize immaterial elements of offer: knowledge, skill, experience, hospitality, etc. Montenegrin agriculture is characterized by small households, unexploited ground, low usage levels of mineral fertilizers and pesticides, inability to use mechanization, water shortage and undeveloped infrastructure makes it uncompetitive in quantitative sense but presents an excellent base for its transformation from conventional to eco farming. Strategic linking of ecotourism with ecological farming presents real chance for sustainable development of rural areas in Montenegro.

**Key words:** ecotourism, ecological farming, sustainable development, rural areas, Montenegro

**Introduction**

Sustainable rural development represents a huge challenge for both developed and underdeveloped countries. With proper development policy in rural areas pressure is starting to decrease in urban areas. It is impossible to envisage development of rural areas through the development of agriculture, as it was done until now, we need a much broader approach. Apart from improvement of competitiveness of agriculture, it implies a diversification of economical activities, promotion of the environment in total improvement of the quality of life of the rural population. Agriculture will still remain the most dominant economy activity of the rural population but at the same time we should develop other complementary activities such as tourism, trade, craftsmanship, etc. Global trends of returning to the unspoiled nature and to healthy lifestyle, growing conscience on necessity for preservation of the environment have created ecotourism as an alternative to mass tourism and eco way of production as alternative to conventional agriculture. New tourists are becoming more and more demanding in every aspect, food as well. They are looking for quality and authentic local products. Research has shown that their expenditures for food and beverages make one third of travel



expenditure in total. In Montenegro there is significant potential for ecotourism development and ecological farming. However, establishing certain relations between these two economy branches is a long term process that requires synchronized action from every stakeholder. This action must be based on principles of sustainable economic development, preservation of the environment and diminishing poverty within the local community. Strategic interlinking of these two activities will provide sustainable management of natural resources and significantly contribute to sustainable development of rural areas.

### **Methodology and objectives**

This paper analyzes views, both domestic and international authors, about the importance and characteristics of ecotourism and ecological farming development in rural areas. The main objective of this paper is to highlight the need and possibilities of the strategic nexus of these branches of the economy, in the function of sustainable development in rural areas of Montenegro. The study is based on secondary sources. The initial hypothesis is: In Montenegro there is significant potential for ecotourism development and ecological farming and their strategic nexus represents a real opportunity for sustainable development.

### **Ecotourism as mechanism for sustainable development**

One of the most significant and the fastest growing forms of selective tourism worldwide is ecotourism. Ecotourism involves creation of synergetic relations between tourism, biodiversity and local community with appliance of adequate management strategy (Ross and Wall, 1999). Honey (1999) defines: „Ecotourism is travel to fragile, pristine and usually protected areas that strives to be low impact and usually small scale. It helps educate the traveler; provides funds for conservation; directly benefits the economic development and political empowerment of local communities; and fosters respect for different cultures and for human rights“.

Ecotourism is a term referring to ecological tourism and this concept of tourism is being implemented all around the world. Montenegro, especially protected areas, are very attractive for ecotourism development. Their biological and cultural diversity represents vast ecotourism potential (Bulatovic D. and Bulatovic A., 2012). When done in the right way, ecotourism is a mechanism for sustainable development. Ecotourism can give benefits to local communities, the benefits of economic and other value-added benefits, such as nature recreation, the value of beauty, aesthetics, scientific values, education and other social values. One of ecotourism's greatest strengths is that it helps to develop better cooperation between the tourism industry and agriculture. Ecotourism can provide a strong economic incentive to small farmers to commit to biodiversity-friendly agriculture management.

It is not always easy for an individual ecotourist to choose between competing products on environmental grounds (Buckley, 1995). Crucial marketing instrument for ecotourism are ecolabels. Marking of quality products with ecolabel is recognized as a way to differentiate quality of product within the meaning of environment protection, health or consummation acceptability. Montenegro, as a country striving towards European integrations, should accept and put in use some of the international ecolabelling programs related to tourism (Gren Globe, EU Ecotourism Labelling standard-EETLS), which will make it easier for Montenegro to obtain recognition as quality ecotourist destination.

### **Ecological farming and benefits for the development of rural areas**

Ecological farming is a growing sector in World. Interest in ecologically produced food is increasing throughout the World. Food consumption patterns are changing as a result of health and environmental issues. Global demand for ecological products remains robust, with sales increasing by over five billion US dollars a year (Willer, Youssefi-Menzler and Sorensen, 2009). According FiBL/IFOAM survey (2009) all over the World there are 37.2 million hectares of ecological farming land. The regions with the largest areas of ecological farming land are Oceania (12.2 million hectares), Europe (9.3 million hectares), and Latin America (8.6 million hectares). The countries with the most ecological farming land are Australia, Argentina, and the United States. Currently 0.9 percent of the world's farming land is ecological. Compared with the previous survey since 2008, growth was strongest in Europe, where the area increased by almost one million hectares. The countries with the largest increases were Turkey and Spain. The countries with the largest markets are the US, Germany, and France; the highest per capita consumption is Denmark, Switzerland, and Austria (Willer and Lukas, 2011).

“Ecological farming“ is a broad descriptor for agricultural methods that seek to be more sustainable than conventional farming by avoiding negative unintended natural and social consequences (Killebrew, Cullelev and Anderson, 2009). The concept of time is central to ecological farming's definition. In reaction to conventional farming's implicit focus on the current period, ecological farming takes a long-term view. By using assets in a way that does not deplete them, ecological farming aims to maintain productivity and usefulness to society in perpetuity (Pretty, 1999; Rigby and Caceres, 2001). Ecological farming is supported in most European countries in the context of rural development programmes (Council Regulation (EC) No 1698, 2005). Most EU27/EEA states have implemented area payments to support conversion to and (in most cases) continued ecological production, with Bulgaria and Romania due to introduce support. However, payment rates, eligibility conditions and requirements vary considerably between countries (Tuson and Lampkin 2006). Some EU member states have even made the objectives of their support explicit, namely, that between 10 and 20 percent of total farm land should be organically cultivated in the near future (Schmid et al. 2008). Consumption of ecological food is 4.5 to 5.5% of the total food market in countries such as Denmark, Austria and Switzerland. However, while the area of ecological land has also expanded rapidly in many new EU member states as well as in candidate and potential EU candidate countries, with annual growth rates of up to 100%, consumption levels have remained very low in these countries (<1%).

Ecological farming practices are ideally suited for poor and smallholder farmers, as they require minimal or no external inputs, use locally and naturally available materials to produce high-quality products, and encourage a whole systemic approach to farming that is more diverse and resistant to stress (UNEP and UNCTAD, 2008). Ecological farming provides employment in poor rural areas without increasing farmer's costs: what she saves on chemicals, she can spend on labour. Lee (2005) and Shiferaw et al. (2009) find that labor availability impacts the adoption of ecological farming techniques, which are typically more labor intensive than conventional methods.

Tirado (2009) considered the main benefits of ecological farming:

- Ecological farming provides the ability of communities to feed themselves and ensures a future of healthy farming and healthy food to all people.
- Ecological farming protects soils from erosion and degradation, increases soil fertility, conserves water and natural habitats and reduces emission of greenhouse gases.
- Ecological farming is both a climate change mitigation and adaptation strategy. Ecological farming can provide large-scale carbon sinks and offer many other options

for mitigation of climate change. In addition, farming with biodiversity is the most effective strategy to adapt agriculture to future climatic conditions. A mix of different crops and varieties in one field is a proven and highly reliable farming method to increase resilience to erratic weather changes.

- Ecological farming both relies on and protects nature by taking advantage of natural goods and services, such as biodiversity, nutrient cycling, soil regeneration and natural enemies of pests, and integrating these natural goods into agroecological systems that ensure food for all today and tomorrow.

Ability of ecological farming to overcome local constraints hinges on the availability of education and training about selecting and implementing sustainable practices. Lee (2005) finds evidence that involving nongovernmental organizations, farmer-based organizations, outreach programs, and extension services in providing information and training to farmers significantly increases the successful adoption of sustainable agricultural practices.

### **Ecological farming in Montenegro**

Eco or organic farming in Montenegro, despite some tangible efforts invested in it during last years, is still at the beginning. In Montenegro there is legal, institutional and strategic frame for ecological farming production that needs to be further developed and promoted. The State through Agro-budget is investing significant but insufficient assets in this area. The Law on organic agriculture was passed in 2004. („Sl. list RCG”, br. 49/04). Subordinate legislation is used to regulate certain aspects of organic productions. These regulations are largely conformed with EU regulations No. 31991R2092 i 31994R2381.

National food production and development of rural areas program for 2009-2013. represents an operative program for practicing agrarian policy and special place within this program is reserved for ecological production. One step further in promoting and supporting represents Action plan for development of organic production in Montenegro, set for the period from 2012 to 2017, this plan was written by Ministry of agriculture and rural development within the “Development program for organic agriculture” (OADP) project, which is being realized with the financial support from the Government of Kingdom of Denmark. Increase in interest for organic production was boosted due to another international project meant for small businesses in ecological farming-FAO project. Agricultural producers had an opportunity to obtain grants through stated projects for investments in ecological farming; trainings were conducted for producers, counselors and inspectors of certifying body. Faculty of Biotechnology has introduced a course in ecological farming for its students.

As a result of stated efforts during the last several years we can see an increase in interest for ecological farming in Montenegro. Whether the needed conditions for establishing ecologic farms are met and whether proper methods are applied is being verified by the Government founded organization named “Monteoragnica”. “Monteorganica” is also in charge for issuing certificates for products gained through ecological farming methods. It is founded in 2005 for managing issues such as control of the quality and providing certificates in ecological farming and it is operating under authority of Ministry of agriculture and rural development since 2006. In control and certification process in 2011 one hundred producers were involved. Surfaces under ecological farming in 2011 constitute 0.6% of plough land in total. Altogether, under organic production 3.068,07 ha of plough land was registered, where of 200,29 ha goes to arable land (farmers crops and cultivated medicinal plants 119,81ha; vegetables 2,29 ha; orchards 75,52 ha; vineyards 2,67 ha) and 2867,78ha are meadows and pastures. Apart from this, more than 139.000,00 ha are used for collecting medicinal herbs and sylvan products (Ministry of agriculture and rural development, 2012).

However, Montenegrin ecological products offer in market is still small, and same could be said for the demand of such products. This can be explained with the fact that process of transition from conventional to ecological farming lasts from one to three years, insufficient subventions, knowledge of production rules and marking of ecological products and with unsatisfactory promotion. Also, problem is low income of the buyers and their insufficient knowledge on benefits of ecological farming. However, it is certain that production of ecological agricultural products in time to come will grow and growing production needs growing market to make it economically sustainable. Experiences of other countries can not be applied directly but can be helpful. In strategic positioning of ecological farming assets gained through IPARD program can be of help, and so can any other opportunity for funding available through the joining process of Montenegro with EU.

The major comparative advantage of Montenegro when ecological farming is in question is suitable and diverse climatic conditions that enable production of various agricultural products, although in limited quantities. In general, Montenegro has got well preserved nature and significant number of traditional products, extensive system of agricultural production prevails with limited appliance of pesticides and fertilizers that makes transition to ecological or organic farming easier. This means that there is a tremendous potential to make significant part of agriculture ecological. In order to make Montenegro to turn its comparative advantages into competitive, certification and control process will come in more than handy. The goal of these processes is to confirm that the product is gained in accordance with the regulations in order to prevent fraud and to build confidence between the producers and the buyers. Further growth of domestic and foreign demand for eco farming products can be achieved through ecotourism, which is an important marketing channel, so it is necessary to conduct intensive activities to create tighter connection between agriculture and this sector. This connotes promotional and educational activities with tourism agents on importance of ecological food on the development and promotion of ecotourism offer and also education of food manufactures as an important part of tourism. Promotion should be based primarily on the interest of the buyers for quality, tasty and traditional food and for Montenegrin ecological products that differ from imported ones.

#### **Strategic nexus of ecotourism and ecological farming-chance for sustainable development of rural areas**

At the end of the 20<sup>th</sup> century the concept of rural development becomes equally important in developed countries and in countries undergoing transition. New policies of rural development are focused on defining efficient mechanisms which would secure coordination of agriculture development and other activities in rural areas in accordance with principles of sustainable development and all for the sake of improving population's life standards and quality (Bogdanov, 2007).

One of the activities that can be used to improve life standards and quality of life in rural areas certainly is ecotourism. The Food and Agriculture Organization of the United Nations - FAO views ecotourism as an opportunity for farmers and/or foresters to generate additional income, and at the same time as one of the most effective ways of preserving biodiversity. The symbiotic relationship between tourism and agriculture as a key element of an environmentally and socially responsible tourism. The best ecotourism project is certainly one that includes the local community, preserves the local environment, aspires towards local ownership, showcases innovative environmental practices and sources food and supplies from the local area.

Therefore, development of ecological farming is tightly linked to the development of ecotourism in Montenegro. It will support ecotourism development while ecotourism in

combination with it will open new jobs and improve life in rural areas. By opening new working positions in rural areas migration of population from rural areas to city centers will decline and so will the pressure on job market. Through connection between ecological farming with ecotourism, besides selling agricultural products, new opportunities for additional income will appear through providing touristic services at the farms. Ecotourists are often very interested in learning about the ways of life and work in local community and are very interested in active participation in performing farm jobs. However, establishing a proper bond between these two branches of economy is a long term process that demands synchronized action from both sides.

### **Conclusion**

Ecotourism has presented itself as the most appropriate form of tourism development in rural areas worldwide, while ecological farming continually replaces the conventional. Rural areas of Montenegro are rich with natural beauty, cultural and historical attractions that represent extraordinary potential for ecotourism development. Also, they represent ecologically pure and diverse space with outstanding natural conditions for the development of ecological farming. However, ecotourism and ecological farming are still at the beginning when it comes to quantity and/or diversity of products while rural areas are poor, encumbered with depopulation and other problems. Strategic nexus of ecotourism and ecologic farming represents a chance to overcome these problems and chance for sustainable development of rural areas. Ecotourists mostly desire to try ecological, traditional and authentic high quality Montenegrin food while local agricultural products are able to provide a quality answer on such demands. In order to unleash developing potential of rural areas with sustainable usage of natural resources strategic approach and synchronized action of every stakeholder on taking various measures for promoting, encouraging and directing ecotourism development and ecological farming is needed. Among these measures, besides subventions from the government, very important role is entrusted to certification and control measures of ecotouristic and agricultural products.

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## **POLICY AND INSTITUTIONAL ANALYSES OF MANGROVE MANAGEMENT IN THE INDUS RIVER DELTA**

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### **Abstract**

Evolving concerns over policy and institutions for the management of mangroves in the Indus Delta of Pakistan are being analyzed in this study. Primary and secondary information was collected through document analysis, in-depth interviews and group discussions. Findings of the policy analysis indicated a lack of clear policies focused on the conservation and management of mangroves due primarily to the conventional wisdom of seeing mangroves as economically less valuable resources. This had resulted in the split ownership of mangroves shared by three agencies namely, Port Qasim Authority (PQA), Sindh Forest Department (SFD) and Board of Revenue. While BoR and PQA were lacking any appropriate institutional arrangements for mangroves, SFD established a relatively management system for their mangroves owing to the agency's primary mandate. Broad suggestions have been given to address policy and institutional issues related to the mangroves.

**Keywords:** Forestry policies, Indus Delta, Institutional arrangements, Mangroves

### **Introduction**

Tropical environments such as mangroves, rainforests and coral reefs are the most fragile and threatened ecosystems of earth. Although mangroves account for a small proportion of the total area of these fragile ecosystems, their loss has surpassed that of the rainforests and coral reefs (Valiela, Bowen, & York, 2001). From 1980-2005, the global loss of mangroves was estimated at about 3.5 Mha (indicating a decrease of 19 percent) of which about 1.9 Mha or 54 percent occurred in Asia (FAO, 2007a, 2007b). The global decrease of mangroves could be attributed to aquaculture for 52 percent, tree cutting for charcoal making and timber for 26 percent, upstream diversion of river flows for 11 percent, and forest damage by application of toxic herbicides, expansion of agricultural land, salt works and infrastructure for the remaining 11 percent (Valiela et al., 2001). While these are the superficial causes of mangrove loss, the underlying causes could be the weak institutional arrangements for the management of these precious resources. For long, policymakers in various parts of the world had ignored mangroves as 'wastelands' available for conversion into the other so called 'economically valuable land uses' (Choudhury, 1997; Hellier, 1988; Huitric, Folke, & Kautsky, 2002; Primavera, 2005). Nevertheless, now mangroves are increasingly being recognized for various productive and protective services to the humankind and has inspired the ecologist and environmentalist lobbies to advocate for the conservation and protection of these ecosystems (FAO, 2007b). To what extent such recognition has been translated into policies and institutional arrangement conducive to sustainable management of mangroves is a matter that needs further investigation. This paper specifically explores policy and institutional arrangements for the management of Indus River Delta mangroves in Pakistan as an attempt to fulfill the aforementioned research gap.

### Research Methods

This study followed a two-step analysis. Firstly, a thorough policy analysis attempted to understand the context within which the existing mangroves governance systems had evolved. Based on foundation of first-stage analysis, the second-stage analysis assessed the institutional appropriateness for the management of mangroves under the jurisdictions of three agencies namely: PQA, SFD and BoR. The information reflecting the opinions of these agencies was collected through in-depth interviews with the concerned officials and, wherever possible, substantiated with group discussions conducted at nine mangroves dependent villages, published and unpublished reports, action plans, and policy and project documents. Supplementary information came from NGO and INGO representatives and other persons with knowledge on institutional and socioeconomic aspects of mangroves management in the Indus Delta. Since mangroves are a kind of forest, they have been governed by forestry policies in Pakistan. Consistent with this, the evolution of mangrove governance is assessed in relation to the forestry policies. In this regard, the intentions of the evolving forestry policies in terms of their concern about mangrove conservation have been analyzed based on four main criteria and associated indicators (4: Framework for ). The indicators for institutional analysis were drawn from the literature on common property resources. Agrawal et al. (2002) and Agrawal et al. (2008) found from the review of a large body of empirical work that most influential factors governing natural resources including forest were: the clarity in stipulation of user rights and duties; greater participation of resource dependent communities; adequate monitoring of resources and resource outcomes; enforcement of property rights; and investments in institutional capacities..

Table 4: Framework for the institutional analysis of mangrove management

Parameters and Indicators	Data type and sources
1. Boundary and property rights in mangrove areas <ul style="list-style-type: none"> <li>- Delineation of boundaries</li> <li>- Managerial staff's familiarity with the boundaries</li> <li>- Field staff's familiarity with the boundaries</li> <li>- Stipulation of property rights in mangrove areas</li> <li>- Stipulation of access and withdrawal rights of local communities</li> </ul>	Qualitative data obtained through field observation, interviews, policy documents
2. Monitoring mangroves use <ul style="list-style-type: none"> <li>- Provision of field staff for monitoring</li> <li>- Enforcement of the laws on the use of mangroves</li> <li>- Authority to take legal action against violators of the rules</li> </ul>	Qualitative data obtained through interviews and FGDs
3. Restoration and conservation of mangroves <ul style="list-style-type: none"> <li>- Efforts taken for restoration and conservation</li> <li>- In-house expertise in plantation and restoration</li> <li>- Staff capacity</li> <li>- Partnership with other agencies for mangrove conservation</li> </ul>	Qualitative and Quantitative data obtained through interviews, field visits and project documents
4. Community participation initiatives <ul style="list-style-type: none"> <li>- Participation in mangroves restoration and conservation</li> <li>- Awareness-raising on the importance of mangroves</li> </ul>	Qualitative information obtained through interviews and FGDs



### **Mangrove Management: Evolving Policy Concerns**

While pursuing the revenue seeking forest policies of the British Asia, the colonial forestry agency introduced silvicultural management systems in mangrove forests of Sunderban in the then British-India and Matang of Malaysia. Nonetheless, the colonial foresters did not pay any attention to the management of mangroves in the Indus Delta because of the perception that these resources had no direct economic value. This perception was further reinforced by the dominance of mangroves by single species of *Avicennia marina* (locally known as *Timir*) characterized by low quality timber, with little economic value. Therefore, the mangrove covered areas of the Indus Delta were categorized as ‘wastelands’ and put under the jurisdiction of the Board of Revenue (BoR). Nevertheless, there are evidences that in the 1946 one of the colonial foresters proposed scientific management of the Indus Delta mangroves (SFD 1985) but such proposal could not be materialized owing to the end of colonial rule in the then British-India. Following its independence in 1947, initially Pakistan continued with the colonial forest policy of 1894 to cope with scarcity of forest products. However, soon the policy proved to be inadequate as it sought preserving existing forests while the forest scarce country was in need of expanding its forest cover.

Subsequently, the Government promulgated National Forest Policy of 1955 emphasizing plantations of fast growing exotic tree species along roads, railways and canals, and on wastelands. This policy proved a landmark policy in the management of the Indus Delta mangroves that were at that time the wastelands of BoR. Given the fact that the wastelands of the Indus Delta were already occupied by mangroves, SFD saw it as opportunity. As authorized by the still intact Forest Act of 1927, in 1958 the Forestry Section of Food and Agriculture Department of West Pakistan declared these mangroves as ‘Protected Forests’ under the jurisdiction of the Sindh Forest Department (SFD). Subsequently, the SFD established a Coastal Zone Afforestation Division (CZAD) and focused its effort on the completion of three main tasks (SFD 1985). The first task was to take over the control of mangroves, which were sources of firewood and fodder for the local communities. With little agitation, the local communities conceded SFD’s ownership of mangroves and as agreed to pay a nominal royalty for the materials gathered from these forests. The second task, which remained mostly unattained during the stipulated period, was the plantation of mangroves on fallow mudflats. The most important task, however, was to collect all necessary information required for the preparation of a working plan for the silvicultural management of mangroves as required by the forest policies at that time. Nevertheless, not much could be done until 1963 for effective conservation of mangroves (SFD 1985).

The first working plan for management of mangroves was introduced in 1963 and was valid till 1983. With scant in-house knowledge on mangroves and their management, the plan was mostly inspired by the Sunderban management plan of East Pakistan (Bangladesh since 1971). In accordance with the forest policies of 1962, the key objective of the management plan was the plantation of commercially valuable exotic mangrove species while other objectives included the maintaining healthy mangrove cover for the protection of coastline, inland agriculture and human settlements from the effects of the sea (SFD 1964). While half way through its implementation, the plan was failed to comply with the policies aspiring mangroves to contribute into economic development of the country. This was because of the fact that *Avicennia marina* species, constituting more than 90 percent of the mangrove cover had a fourth grade timber (see mangrove wood classification by Becking et al. 1922 in Chapman 1976) that had a utility only as poor quality wood fuel. Besides, the transportation of wood from creeks through boats was also uneconomical. In some cases, the cost of tree

felling and transportation was more than twice the revenue from the mangroves. This led towards the abolition of CZAD in 1975 (SFD 1985). Until then standing trees used to be sold to the contractors who would bear all cost incurred on the harvesting of timber. As a last resort, SFD created a Mangrove Forest Utilization Wing for absolute departmental exploitation. However, this experiment also proved futile, thereby abandonment of the policy of commercial utilization of mangroves (SFD 1985).

In the 1970s the government established the Port Muhammad Bin Qasim to foster the international trade. To facilitate the establishment of the port, the Government asked SFD to transfer an area of about 64,000 hectares covered with mangroves to the Port Muhammad Bin Qasim Authority (PQA). The SFD had no alternative other than complying with the order, which was reinforced by the financial realities of mangrove management. Besides, perpetuation of the British-Indian Forest Policy (1894) urging FDs not to hesitate in relinquishing any forestland for more valuable land uses, such as agriculture, tacitly facilitated the transfer of mangroves to PQA in 1973. Apparently, there was virtually no policy concern about the ecological, aesthetic and social value of mangroves. While thousands of hectares of transferred mangroves were cleared for the construction of the port, the remaining ones were overseen by PQA as protected forest.

Not much effort was made for the management of mangroves until 1985 when SFD prepared their second working plan (1985–2005) for the remaining mangroves under its jurisdiction. This plan envisaged that through better understanding of the value of mangroves, the desired objective of making considerable contribution to the forest wealth of the country would be achieved. Although, the key objectives of this plan were similar to the previous plan, it also compared the protective value of mangroves with their productive value (SFD 1985). Besides, attention was also paid on raising public awareness about the indirect benefits of mangroves. The plan was also concerned with the sustainable supply of firewood and fodder to the local communities. A new Coastal Forest Division (CFD) was established to pursue the objectives of the second plan. Reportedly, plantation of mangroves was also carried out during this period (IUCN Pakistan 2005). Since, the expiry of the second working plan in 2005 till time this survey conducted in 2010, no new working plan was either released or was under preparation.

Meanwhile, Pakistan was also developing a massive irrigation network on the Indus River comprising several large dams, reservoirs and canal networks. Upon the completion of the major phase of the irrigation development in 1970s, the most of the river flow regimes were diverted for upstream agricultural. This caused severe water shortages in the downstream part of the river. As, a result, the agricultural mudflats of the central delta (Keti Bandar and Kharo Chan sub-districts) could no more facilitate the cultivation of red paddy and were ultimately abandoned. In the absence of freshwater, these mudflats were taken over by tidal inundation and were ultimately abandoned by the local communities (Memon and Thapa 2011). Then, the prolonged fallowing of erstwhile rice fields provided an opportunity for the propagation of mangroves which by default came under the jurisdiction of BoR. Three agencies namely: SFD, PQA and BoR thus claim an area that contains more than 90 percent mangrove cover of the country (Memon 2011).

Besides understanding the fragmented ownership patterns of the Indus Delta mangroves, the review of forest policies reflect few other important themes which have important insights for understanding the existing institutional arrangement for the management of mangroves. First, it is pertinent to note that throughout the evolution of forestry policies in Pakistan, mangroves

were exclusively stated first in 1962 and later has been a continuous theme of various policies only since the adoption of NCS in 1992. The revitalized interest in mangroves is understandable in the context sustainable development paradigm that stated gaining worldwide popularity in the 1980s. Second, despite of the failure or weak implementation of almost every forest related policy released since the independence of Pakistan, a blind faith that the FDs could improve the state of country's forests has been the most durable theme of various manifestos. Even the influence of international development thinking and donors prescribing community participation appeared since the policy of 1991 (Ali 2009; Babar et al. 2007) and NCS 1992 could not pose any serious challenge to the domination of FD over forests. Subsequent forest policies of 2001 and 2007 and the environmental policy 2005 demonstrate more relevance in terms of the importance of mangroves and their management. Nevertheless, their effectiveness is limited as both forest policies of 2001 and 2007 are still drafts awaiting the cabinet approval while the environmental policy 2005 provide overarching theme that could have some effect only if translated into concrete actions.

## **Institutional Arrangements for the Management of Mangroves**

### ***Boundary and Property Rights Issues in Mangrove Areas***

The boundaries of mangrove areas under the jurisdictions of PQA, SFD and BoR were clearly drawn mostly following creeks in the deltaic landscape. In the areas where such creeks were absent, concrete pillars were erected by SFD in 1962 to prevent any encroachment for agriculture and human settlements. Later when SFD realized this was unlikely to happen primarily due to the unavailability of freshwater in mangrove dominated land, they stopped maintenance of those pillars, most of which had already disappeared. In the case of mangroves under BoR, their boundaries were drawn with reference to various natural and manmade features such as creeks, canal network and roads as these lands were settled. Not only the sub-district level revenue officials but also many of the local people could easily identify the boundaries in BoR area. However, the PQA and SFD officials were not much familiar with the otherwise very clear boundaries of mangroves under their jurisdictions because of their limited field visits and frequent transfers.

Land rights in the areas under SFD and PQA were quite straightforward since these lands were government property and thereby devoid of any private claims. However, land rights in BoR area were somewhat complex as it was gathered based on ZDA (2011) that about 25 percent of the land in Keti Bandar and Kharo Chan sub-districts was under private property rights arrangements (locally called *Qabooli* lands) and another five percent was reserved for public purposes including the land under sanctioned villages, roads and canal networks. The remaining two thirds of the land were registered as government property (locally called *Na-Qabooli* lands). Similarly, rights of the local communities to access and withdraw mangrove forest products from the areas under SFD and PQA were rather clear due to their status as protected forests. Legally, in a protected or second-class forest, local people could collect forest products for their domestic use, while the collection for commercial purpose was prohibited.

Regarding the mangroves of BoR, there was not much concern about the access and rights as this agency was not primarily responsible for the management of mangroves. Despite such limitations on the part of BoR, the local people avoided cutting these mangroves for wood fuel reportedly because the required amount of dead, dying or fallen trees was still unavailable in relatively young mangroves in the BoR area. Therefore, the local people living in BoR area

also continue to collect firewood from the mangroves of Dabbo, Chan and Kajhar Creeks, which is actually the area under the jurisdiction of SFD.

### ***Monitoring the Use and Condition of Mangroves***

All three agencies had a provision of field staffs, but only those appointed by SFD were formally responsible for monitoring the local use of mangroves. However, the monitoring of SFD's mangroves could also not be carried out effectively due to inadequate number of field staffs and logistics such as patrolling boats. Moreover, there were legal provisions of fines and sanctions on the reckless or illegal use of mangroves, but reportedly the cases of arrest and prosecutions against rule violators were very rare. It was observed at various group discussions that there was an unofficial consensus among the camel herders and SFD officials that the former will never graze their camels in the planted mangroves, while the latter will not strictly prohibit the grazing of camels in the naturally grown mangroves. In the case of PQA, the concerned official reported that although nobody was formally assigned such a responsibility but the security guards were monitoring the use and status of mangroves as a part of their routine watch on the assets and infrastructure of the port. However, PQA guards were reportedly not much concerned with the permissible use of mangroves as reflected from group discussions in PQA area. Since BoR had not specified any set of rules and regulations on the local use of mangroves, these resources were in principle open for use by everybody. Again, this was attributed to mangroves conservation not being the primary responsibility of BoR.

### ***Restoration and Conservation of Mangroves***

SFD has pioneered the restoration and conservation of mangroves in the Indus Delta and has replanted nearly 20,000 hectares of mangrove in discrete locations (IUCN Pakistan 2003). At a smaller scale, PQA has also supported the plantation of about 1,200 hectares of mangroves (IUCN Pakistan 2000; WWF Pakistan 2003). Most mangroves planted by PQA were in replacement of those cleared for the development of port facilities including new jetties. On the other hand, BoR had not initiated any mangrove plantation in their area, as they did not consider it as their responsibility. With an underlying objective of enhancing the financial viability of mangroves, SFD had made significant attempts to reintroduce the extinct or exotic mangrove species in the Indus Delta and to some extent had been successful in the reintroduction of *Rhizophora mucronata* species. In the past attempts were also made to introduce commercially important exotic species such as *Excoecaria agallocha* and *Nypa fruticans*. However, since the survival rate of these species was very low, the policy of promotion of such species was abandoned while currently all plantation projects plant *Rhizophora mucronata* species as it can be easily grown in the local environ. SFD has got the in-house capacity including human resources with good experience in mangrove plantation, while the other two agencies have not. PQA has to some extent overcome this by involving some local and international NGOs having the capacity in mangrove plantation. None of the agencies, including SFD, had made any investment in institutional capacity building for mangrove conservation and restoration. SFD realized such need but, reportedly, lacked required financial resources; while PQA and BoR did not realize such need considering it as beyond their mandates. Almost all mangrove plantations in the Indus Delta had been accomplished through the partnerships with regional and international agencies such as the World Bank, IUCN and WWF. Particularly, SFD has a long established partnership with foresaid agencies while enduring mangrove conservation and restoration (IUCN Pakistan 2005). PQA had also collaborated with NGOs like Shirkat Gah, WWF and IUCN to carry out

mangrove plantation and conservation. However, BoR had not carried out any such activity either independently or in collaboration with other agencies.

### ***Community Participation in Mangrove Management***

None of the three agencies had made any kind of arrangement mustering local involvement in management of mangroves under their respective jurisdictions. In the case of PQA and BoR, obviously it was because these agencies had no formal mandate for mangrove conservation. Surprisingly, despite its weak institutional capacity mentioned above, SFD also had no arrangement for involving the local communities in the management and conservation of mangroves. SFD had simply prepared a list of mangrove dependent communities for the entire Indus Delta in 1998 (Shah 1998) but the list was never utilized for any meaningful purpose and is already outdated. Nonetheless, the officials of SFD and PQA claimed that they had involved the local communities in the restoration and conservation of mangroves. However, a follow-up discussion revealed that the so called participation was limited to the engagement of local people in mangrove plantation as wage laborers. Beyond this, none of the agencies had any mechanism through which the local communities could be mobilized to participate in the conservation and management of mangroves or carryout the registration of mangrove user groups. The discussions held with concerned officials gave an expression that social mobilization, mangrove forestry extension, formation and registration of mangrove user groups were alien concepts alien for these agencies. Similarly, none of these agencies had any enthusiasm to introduce any participatory mangrove management systems that current policy framework since adoption of NCS 1992 advocates.

### **Discussion and Conclusion**

The wisdom of 20<sup>th</sup> century emphasized on the ‘ownership’ of common pool resources such as mangroves and forests as an important condition for their sustainability. Over the last few decades, such understanding has resulted in retreat of governments’ ownership of forests in favor of the private and community forestry (Webb and Shivakoti 2008). Nevertheless, in a situation where governments still own about 80 percent of the global forests (FAO 2010), it is important to ask weather all forests under the ‘umbrella of government ownership’ are the responsibility of agencies appropriately mandated for the sustainable management and conservation of these resources. Findings that mangroves in the Indus Delta are undergoing varying degrees of management and conservation thus corroborate with the emerging consensus that effectiveness of any resource management is only partly explained by who owns them (Agrawal et al. 2008; Schlager and Ostrom 1992). The study further adds that only the owners, managers or users with an objective of sustainable conservation and management of their resources are likely to establish effective resource governance systems. Without the interests of the concerned owners or managers, resources will suffer from the lack of interests and remain vulnerable to unsustainable utilization and eventually degradation no matter under any kind of property right arrangements.

Various implications emerge from this study to guide policy and institutional arrangements for the sustainable management of mangroves in the Indus Delta. Learning lesson from the past fallacy, a robust policy clearly highlighting the need for concentrated national, regional and local level efforts for effective management of mangroves should be devised first. In this context, mangroves should be considered as one of the precious ecosystems comprising not only forest, but also fishery and other allied biotic resources, and providing ecological services as well as many socioeconomic benefits. In view of virtually “no or weak

management system” in all areas arising primarily from the split ownership of mangroves, ideally, it makes sense to suggest integration of all mangroves in the Indus Delta under the jurisdiction of a single agency. Such an agency could be SFD as its primary responsibility is to conserve and manage forest resources including mangroves, and has built some in-house capacity to discharge its responsibilities. As the findings of this revealed, SFD’s capacity to discharge its responsibilities is far from satisfactory, this would require investments in equipping the agency with required trainings, resources and manpower to accomplish this task. Provided this option fails to garner needed support, PQA and BoR should also be obliged to take responsibility for management and conservation of mangroves by introducing separate mangrove conservation wings in their organizational landscapes. Otherwise, mangroves under the jurisdictions of these agencies would always remain vulnerable. This should be followed by building mangroves conservation and management capacities of these two agencies and strengthening the existing capacity of SFD through the provision appropriate trainings, resources and manpower.

While local communities are utilizing mangroves to meet some of their basic needs including fodder and wood fuel, their participation in the management of mangroves is lacking. In view of this reality combined with the lack of adequate required resources, including the manpower, on the part of mangrove owners, provision of an appropriate institutional mechanism mustering local community participation in sustainable management of mangroves is desirable. Such mechanism should enable the local communities to act as custodians of mangroves while continuing the use of these resources for fulfillment of their basic needs. Studies elsewhere suggest that community participation in natural resources management has emerged as a powerful institutional arrangement particularly where governments lack capacities and funds required to conserve and manage such resources effectively (Webb, 2008). Though so far Pakistan has not yet been able to adopt a policy of community participation in natural resources given the provisions in NSC 1992 regarding community forestry for second class forests, the agencies governing mangroves can take action towards this direction as it would ensure effective management and conservation of mangroves.

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**TYPE OF FARMING AS A FACTOR DIFFERENTIATING THE LEVEL OF FARM SUPPORT UNDER COMMON AGRICULTURAL POLICY IN POLAND**

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**Abstract**

The article presents the differentiation range of the EU support level gained by Polish agricultural farms dealing with different types of farming. An important aspect of the analysis was also to determine the effect of the direct support to the income situation of agricultural producers. Total subsidies on current operations, particularly including direct payments and the subsidies designed for the development of rural areas, were analysed in detail. Their amount was considered in relation to the farm income.

The analysis of payment distribution enabled to show which types of farming in the European Union are being supported in the first place. On this basis an attempt was undertaken to find the answer whether the EU farms support system is effective and justified.

Poland's membership in the EU gives rural farms the opportunity to improve their economic situation. The direct support was the main factor of determining the economic status of farms. However, strong differentiation of the amount of this support, as well as farm income dependent on farm specialization in production was noticed. As a result, there is still large number of small farms in which the revenues received by farmers are not sufficient enough to assure them sufficient life standard. Therefore such farms are not able to both develop and invest. Such opportunity have only economically strong and market oriented farms with high production potential, that in the future will determine the position of Polish agriculture in international markets.

**Key words:** type of farming, direct support, farm income, CAP

**Introduction**

Farms functioning on the area of the European Union are classified on the basis of two main criteria: type of farming and economic size. The division was established by the decision of the European Commission on 7th of April 1978. The European Commission regulation act signed on 19<sup>th</sup> of April 2004 refers to the countries which joined the EU in 2004 (Marcysiak 2009).

Type of farming is one of the main determinants of the characterisation of farms in the Community Typology for Agricultural Holdings in the EU. It reflects the system of production predominant in a particular farm and it is determined on the basis of the share of production activities carried out by the farm in the generation of the total value of the standard gross margin (SGM). Due to this classification it is possible to compare the structure of the analyzed farms and their economic situation. (Skar y ska i Zi tek 2006). The type of farming makes a crucial criterion for the classifying of agricultural farms used to prepare reports describing the activities of farms functioning on the area of all EU member states. (Kondraszuk 2009). In Polish FADN data systems even general types of farming are distinguished<sup>37</sup>, which are a combination of 17 basic and 50 detailed types.

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<sup>37</sup> Polish FADN system distinguishes seven types of farming: field crops (AB), horticulture (C), permanent crops (E), milk production (F), grazing animals (G), grainvores (H), mixed production (I).

Upon Poland's accession to the European Union Polish agriculture and rural areas have been covered by the Common Agricultural Policy (CAP). Agricultural producers gained access to new sources of financing to enable not only the current production activity, but also to the restructuring and modernization of farms.

Some part of the funds available basically under structural funds is directed for the development of rural areas. Nevertheless, the most vital form of the support are direct payments, which major role is both the stabilization and ensuring the proper level of agricultural producers income. (Wieliczko 2007). Farms should get high income, which, together with loans and subsidies, will be able to fund the necessary projects, allowing to maintain and increase their competitive position in the future (Krzyanowski 2009).

### **Materials and methods**

The analysis used the relevant literature and secondary data from a sample of farms<sup>38</sup> covered by the FADN (Farm Accountancy Data Network). The scope of the analysis covers the year 2010. The collected data were analysed with the use of descriptive method. The results are presented in tabular form and depicted by graphs.

The basic economic category used for the research were total subsidies on current operations linked to production (not investments) covering most categories of transfers of aid to farms under the CAP, including direct payments and subsidies for the development of rural areas. The second variable was the income from a family farm, which is the economic surplus obtained in the course of farm's operations. It is a reward for a farmer for engaging own production factors in the production process, i.e. labour, land and the capital.

### **Results and discussion**

#### Characteristics of the analysed farms

A representative sample of Polish FADN amounted to approximately 11 thousand entities (farms) conducted different types of agricultural activities. The predominant type of production was mixed production (plant-animal), implemented by 35% of all farms. Next, in terms of numbers, were dairy farms and those specialised in field crops, representing around 20% of the analysed population. The percentage of farms representing other types of farming was relatively lower and amounted respectively to: grainvores (14.5%), other grazing livestock (4.5%), permanent crops (4.8%) and horticulture (4.7%).

The direction of production turned out to be one of the key factors differentiating both the area and economic size, as well as farms production results (tab. 1). In 2010, the analysed acreage of an average farm was 19.2 ha of agricultural land, while the average value of production reached PLN 101 thousand. The largest agricultural area characterised farms focused on field crops. Relatively large acreage belonged also to dairy farmers and farms keeping grainvores. Definitely the smallest were farms of horticultural type.

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<sup>38</sup> FADN is a representative study, which means, among others. that studies are conducted on a random sample of farms. In 2010, after consultation with the European Commission Polish FADN sample size is set at 11 191 households.



Table 1. Farms characteristic according to the type of farming, average values per farm

Specification	AB	C	E	F	G	H	I
Number of farms	2 043	418	427	2 319	487	1 629	3 868
Agricultural land area (ha)	49.9	5.8	9.1	21.0	17.3	19.4	16.1
Economic size (ESU)	26.6	33.2	11.9	22.8	12.9	40.2	14.7
Agricultural production (thous PLN)	166.3	175.8	105.2	116.2	48.9	190.1	72.7

AB- field crops, C- horticulture, E- permanent crops, F- dairy farm, G- rother grazing livestock, H- grainvores, I- mixed production.

One of the major parameters to evaluate the economic situation of the EU's farms is economic size. It is determined on the basis of the total standard gross margins of all activities carried out by the farm. One ESU in 2010 corresponded to EUR 1.200 (Goraj et al. 2011) Among the analysed population economically strongest were farms keeping grainvores and horticulture farms. However, low economic size characterised the entities focused on permanent crops and keeping rother grazing livestock.

The development of a farm depends largely on the ability of producers to improve the efficiency and productivity of the conducted activity and on from the possibility of systematic increase in the level of income (Ginter 2011). The main part of the income generated by farms creates the value of agricultural production. Its highest level calculated per one farm was typical to entities focused on grainvores, field crops and horticulture. The production value of these holdings amounted on average to PLN 177 thousand. and was higher than the average of the FADN field of observation (PLN 101 thous.) by more than 75%. However, the lowest value of production was recorded in farms keeping other grazing livestock and aiming at mixed production.

The level of family farm income received by farms of the agricultural type is determined, not only by the level of costs incurred and the value of production, but also by the profitability of the production, its intensification and the degree of relationship of the entity and the market.

AB- field crops, C- horticulture, E- permanent crops, F- dairy farm,  
G- rother grazing livestock, H- grainvores, I- mixed production.

Figure 1. Average level of family farm income according the type of farming in 2010

The level of income generated by the entities of the various types of farming was strongly diversified. In 2010, the highest income was typical to farmers specialising in horticulture and it amounted on average to PLN 53 thousand per farm. Farms keeping grainvores and dairy farms also achieved a high level of income. All these types of entities are characterised by a relatively high intensity of production and high market competitiveness of manufactured products. On farms with a predominance of field crops and permanent crops the value of income was slightly lower. The lowest income from current operations characterised farmers specialising in mixed production and other grazing livestock. In this group of entities, usually characterised by low profitability of production (including a low ratio of price to the unit costs, e.g. of cereals) and low levels of the processing, the average income per farm does not exceed PLN 30 thousand (fig. 1).

The scope of farms support with EU funds and their impact on income

The Common Agricultural Policy of the European Union is governed by the two pillars. The first one is aimed at supporting the agricultural sector through direct payments and market support. The second comprises the mechanisms influencing the development of rural areas through the structural funds.

AB- field crops, C- horticulture, E- permanent crops, F- dairy farm,  
G- rother grazing livestock, H- grainvores, I- mixed production.

Figure 2. Average value of total subsidies according to the type of farming in 2010

In the analysed period, the average value of total subsidies on current operations on one farm in the FADN field of observation amounted to PLN 21 thousand. Of all the groups of farms, the highest payments were obtained by beneficiaries specialising in field crops (PLN 52 thous.), as well as milk production and herbivores (average PLN 22 thous.). The lowest payments (less than PLN 8,5 thous.) per farm were received by farmers specialising in horticulture and permanent crop. Payments on farms with a predominance of mixed production and keeping grainvores remained at a level similar to the average for the whole community (fig. 2).

Direct payments have a very important position in the structure of the EU support. It represents nearly half of the whole amount of total subsidies on current operations. Poland at the time of accession to the EU decided to implement the Single Area Payment Scheme (SAPS). It consists of two categories of payments. The first is the single area payment due to the land maintained in good agricultural condition. It is awarded to all agricultural producers having agricultural land with an area of not less than one hectare. The second category is made up of complementary payments in the form of subsidies to the area of specific crops (including: cereals, oilseeds, protein, industrial and tobacco plants). Since 2010 farmers can also apply for the so-called special support in the form of payments for legumes and payments for cattle and sheep.

The level of direct payments to agricultural land in the analysed farms in 2010 on average amounted to PLN 10 thousand. Per 1 ha of agricultural land it was PLN 521. Type of farming was a factor differentiating the level of direct payments to agricultural land in the analysed farms. This was mainly due to the significant differences in terms of area of farms and the scale of access to the complementary payments applied to the surface of certain crops and livestock. Farmers specialising in field crops received the highest support on account of direct payments. Per one farm it was more than 2.5 times higher than the average for the whole group. The amount of subsidies similar to the average level was awarded to beneficiaries producing milk and keeping rother grazing livestock. In these three groups of entities agricultural production is mainly based on the acreage of arable land and grassland. In addition, those farms are entitled to separate payments for cows and sheep. The lowest benefits were granted to beneficiaries specialising in permanent crops and horticulture. They were only a third of the average value of subsidies for farms from the FADN field of observation. Such a low level of support was associated with the inability to receive the supplementary subsidies to both the soft fruit plantations and fruit crops.

Another important element of the impact of the CAP on Polish farms is support directed towards the development of rural areas. It includes: agri-environment payments and payments to less-favoured areas (LFA)<sup>39</sup>. Their share in the whole amount of total subsidies in 2010 amounted to 23%. Also with regard to this category of subsidies, there was a visible difference in the amount of support received in particular groups of farms. Average amount of subsidies for rural areas development in the analysed year amounted to PLN 4.7 thousand per farm. As in the case of direct support the highest payments to the development of rural areas was granted to farmers specialising in field crops (PLN 8.1 thous.), while a relatively high to

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<sup>39</sup> In the EU, less-favoured area (LFA) is a term used to describe an area with natural handicaps (lack of water, climate, short crop season and tendencies of depopulation), or that is mountainous or hilly, as defined by its altitude and slope.

beneficiaries specialising in other grazing livestock (PLN 7.3 thousand.). However, the lowest level of support (less than PLN 3 thous.) was reported in case of horticulture farms, as well as farms focused on permanent crops. In other groups of entities the amount of payments for rural development was similar to the average of the FADN field of observation.

The payments received under the CAP is not without significance for the income situation of Polish farms. In the years 2004-2010 the average income of the average farm increased almost two-fold (from 21 to 36 thousand PLN ). With Poland's accession to the EU the factors affecting the level of income of agricultural producers changed significantly. In addition to usual factors such as the volume and value of products, the level of expenditure incurred in the production, as well as the ratio of agricultural prices (the so called "price scissors") there appeared non-market financial support in the form of direct payments and funds from structural programmes. In the long run the income situation of farmers is determined by the production potential of a farm and effective management of available resources (Józwiak 2011). Dynamic growth in the level of total subsidies to farmers under the CAP, resulted in a gradual increase of their income-producing role in the sector. In the years 2004-2010, the average amount of these transfers per one household increased 8-fold (from 2.6 to 21 thousand PLN), and the average share of income from agriculture increased from 13% to 60%.

AB- field crops, C- horticulture, E- permanent crops, F- dairy farm,  
G- rother grazing livestock, H- grainvores, I- mixed production.

Figure 3. Share of total subsidies in the family farm income in 2010

In the case of holdings grouped by type of farming, the role of subsidies in income generation depended mainly on the possibility of gaining complementary payments by the given group of farms, which are awarded to specific production activities. For this reason, the highest (80-96%) ratio between subsidies and the income belonged to farms keeping rother grazing livestock and with a predominance of mixed production. These farms, despite the large surface of agricultural land, were characterised by relatively low value of income and profitability of production, as well as weak links with the market.

A different situation concerned farms strongly related to the market, characterised by higher efficiency and productivity and relatively low use of land as a factor of production. These companies are mainly horticulture farms and dairy farms and those keeping grainvores. It should be noted that, despite the possibility of obtaining single area payments and supplementary payments, the economic situation of producers keeping grainvores is primarily determined by their market activity, and to a much lesser extent, external support (fig. 3).

### Conclusions

To sum up the discussion the following conclusions can be drawn:

- The type of farming in the European Union forms the basis for the classification of farms next to the economic size. It is also an important factor differentiating between the amount of support received by farms and funds under the CAP.
- Among the various external support mechanisms direct payments are the most important one. With regard to farms covered by the FADN they account for almost half of the total subsidies on current operations.
- Most of the funds coming from direct payments went to farms specialising in field crops, and the smallest amount to entities focused on horticulture and fruit production. Differences in access to the EU support derived primarily from highly differentiated size of the analysed farms. The amount of direct payments in individual types of farms was also differentiated by the possibility of receiving supplementary payments.
- The analysis showed a beneficial effect of the CAP mechanisms on the formation of financial situation of individual farms. Among all the appropriate instruments to support agriculture in the European Union a key role in creating the income of farmers, their stability and mitigating the diversification of profitability of agricultural production is played by direct payments.
- The close relationship between agricultural producers income and the dominating type of farming has been noticed. The type of farming also has an important impact on the share of total subsidies in creating family farm income.

Looking at the distribution of support in the agriculture in 2010, one should note that it reflects very well the conditions characteristic of Polish agriculture, as well as the specificity of the EU support system. On the one hand, the majority (more than 75%) of support went to a large number of small farms mainly related to the plant production i.e. the units that in the long-run are not able to develop, regardless of whether they use support or not. They were mostly economically weak farms, characterized by a low association with the market and competitiveness. For this reason, the economic situation was largely dependent on the support of the EU. Moreover, even the increased access to subsidies didn't change their weak position

in the food chain. This implies that the EU support will never be able to fully offset the effects of small-scale production, or insufficient efficiency and productivity of production factors (Czubak et al. 2008). This situation may raise, at least, some questions about effectiveness of support system.

At the other extreme, there was a relatively small number of farms (primarily related to livestock production and horticulture) receiving very high benefits, but their share in the total sum of support to the sector was relatively small. This group of economically strong and market oriented holdings was characterized by high production potential and a relatively small share of total EU support in farm income. Furthermore, these farms will determine the market supply of agricultural products in a much larger extent and food in the country and in the future will determine the competitiveness of Polish agriculture in international markets.

It can therefore be argued that the CAP as such, due to high agrarian fragmentation of majority of Polish farms, only applies to a small part of our agriculture, at least in terms of improving the income condition of farms, efficiency of agricultural activity and competitiveness of agricultural sector. In relation to the remaining part farms it plays a rather social role (Judzińska and Łopaciuk 2012).

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**THE STUDY OF FOOD WASTE IMPACT ON THE HOUSEHOLDS' SOCIO -  
ECONOMIC STATUS IN THE MUNICIPALITY OF VRŠAC**

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**Abstract**

Food waste made by consumers' uneconomical behaviour has negative ecological effects and it also affects households' financial costs. Wasted food, as a consequence of the incomplete use of its edible part, is an important issue and is not sufficiently explored in Serbian science. At a global level it can be claimed that there is a lack of an adequate estimate of the total amount of uneconomically wasted food, a lack of data about food loss' critical stages in the food supply chain, and also about various consequences, that is, possible effects of such waste on ecological, economic and social environment and position of households.

Republic of Serbia is facing increasing problems of such uneconomical food loss (and, thus, resources), although its standard of living does not allow such daily behaviour. In theoretical and methodological terms, there is a problem of quantitative estimates of food waste, made within a household, as well as finding ways for its reduction. The aim of this paper is to analyze the awareness, attitudes, behaviour and ways of treating food and food waste within a household, based on a survey research of a random sample of a one hundred respondents. The results indicate the situation in this field and the patterns of daily behaviour of households in Vrsac municipality, and also estimation of monetary value of the food which is lost hereby. Based on these results, the authors conclude on the relevance of food waste problematic in Serbia and in regards to cultural habits and neglect of economic values of wasted food by surveyed households.

**Key words:** food waste, consumers, household, behavior, economic value

**Introduction**

Food waste arising as a result of poor food handling at different stages of the entire process of food supply is a global issue because of the impact on quality, food safety, economic development and the environment. Historical and cultural heritage, the level of economic development, social structure and the degree of social consciousness lead to a significant difference in the amount of wasted food, and consequently to different approaches to resolving this issue, in comparison to other countries. In middle and high-income countries the largest amount of food is wasted at home, which is usually associated with the consumers' behavior and habits (the losses exceed 40%) (Gustavsson et al., 2011). In low-income countries, a significant loss of food occurs during the early and middle stages of the food supply chain (40% of food loss occurs during harvesting and post-harvest, while much less food is thrown away at the consumer level) (Ibid.). The reason is that consumers in undeveloped countries, which are often subject to unstable political and economic climate, with high rates of poverty, limited income and high unemployment rate, have a strong awareness of food supply uncertainty. Therefore consumers are forced to better plan buying, storing and preparing of food.

Today, the amount of food produced in the world is more than enough to feed the entire population. However, according to FAO, about one third of the food produced globally is thrown away or wasted, which is a significant amount of 1.3 billion tons (Ibid.). Food waste

occurs during the entire food supply process, from the beginning of agricultural production to final consumption in households (Ibid.).

Food waste, as such, represents the loss of economic value for the stakeholders in the supply chain (FAO, 2012). Different studies indicate that a large amount of food is thrown away while still at the stage in which it could be completely used. According to Waste & Resources Action Programme record, in the UK most of the thrown food (4.1 million tones or 61%) is avoidable and could have been eaten if it had been managed better (Ventour, 2008).

Regardless of the achieved level of political and economic development and modernization of a society, the waste of food should be kept to a minimum. Many initiatives have been launched around the world to resolve this issue. In 1974, at the World Food Conference, the reduction of food loss is for the first time offered as a solution to reduce hunger in the world, while in January 2012. EU Parliament adopted a resolution to reduce waste by 50 per cent by 2020 and to improve access to food for EU citizens in need (Segrè 2013: 23).

Serbia is also facing a growing problem of food waste, although the standard of living does not allow that kind of loss. Generally, institutions in Serbia have insufficiently addressed the problem of quantification of food waste, but also of food losses and causes of losses at various stages of the supply chain, as well as the issue of the necessary financial resources for prevention of food loss. Certain phases of food loss have been however researched in domestic science, such as loss at the production, postharvest and processing stages, but very rarely food waste has been investigated. Further research in food waste (that occurs during retain and consumption - thrown food) is essential bearing in mind that food safety is a major issue in the economic, social and environmental terms. In this paper we analyze data from empirical research that aimed to survey consumers' attitudes and behaviour during different stages of daily food management, in households from buying, storing, preparing to food disposal. An important part of this paper is establishing the level of respondents' knowledge and awareness of causes and consequences of wasted food, as well as the level of their will to change behaviour when managing food in households.

### **Materials and methods**

The aim of this paper is to analyze data gathered in empirical research on ways to handle food and food waste at household level through the analysis of awareness, attitudes and behavior of consumers, taking into account the different types of households. The basic assumption is that there is an increasing problem of handling food waste, as well that there are significant effects of such waste on ecological, economic and social environment and the position of households. Based on the available data and the results of other analysis, we have defined the methodology and the sample on which the field survey was conducted in the municipality of Vrsac in the first half of 2013. As a model for the development of the questionnaire we used methodology of *Food waste avoidance benchmark study*<sup>40</sup> that was adjusted to the needs of our research. The field research was conducted by survey of 100 respondents, who are mostly equally responsible for the buying, preparation, storage and disposal of food within their households. The questionnaire covered urban and rural, agricultural and non-agricultural multi-member households in the municipality of Vrsac. In addition to data on demographic, economic and social characteristics of households, questionnaire also contained information about the daily food handling at the monitored households in the Municipality of Vrsac, and the respondents' evaluations of the cash value of food that has been lost by their non rational treatment. The first part of the questionnaire related to socio - economic structure of a household, in order to determine the level of education, sex, place of residence, household type and its economic status. The second part of the questionnaire contained a set of questions that examined consumers' attitudes toward food waste as well as the consumers' conceptual distinction among the categories of food waste. The third part examined the knowledge and

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<sup>40</sup> <http://www.lovefoodhatewaste.nsw.gov.au/portals/0/docs/11339FWABenchmarkstudy.pdf>

attitudes of respondents in relation to food and its possible economic impacts, behaviour of participants during the planning, buying, preparation and storage of food, and an assessment of the economic impact of wasted food on the household budget.

### Results and Discussion

The study conducted in the municipality of Vrsac mostly included non-agricultural urban households (86%) with satisfactory financial situation (84%). Most respondents mainly in charge of the food management at households were female (80%). More detailed analysis of socio-economic features and their intersection with the level of knowledge, awareness, attitudes and behaviour of respondents is very important and it is still pending. However, in this paper we present only the results of the other segments of the questionnaire, which analyzes the attitudes of consumers about food waste, as well as their conceptual knowledge and differentiation between food waste that can and (or) cannot be avoided.

*Knowledge regarding the food waste.* The aim of this section was to analyze the respondents' attitudes about the type of food that they consider to be the waste which can and which cannot be avoided. The results indicate that the respondents think waste that could be avoided by better managing include food such as old frozen food (77%), damaged fresh products (52%), food leftovers on plates, occurring after meals (56%), unfinished drinks (63%), uneaten portions which remain after meals (58%), and packaged food with the expiration date (58%). Under the waste that cannot be avoided, regardless of food management, respondents include fruit and vegetables peels (53%), bones (59%), tea bags or coffee grounds (80%). Given that just over half of respondents correctly classified food waste into a particular category, it can be concluded that a significant amount of waste just comes from lack of knowledge and improper food handling by consumers. Given that the visual appearance has a rather strong influence on average consumers' perception of food, damaged products and unappealing food leftovers are often the reason to avoid consuming, and therefore creating food waste. Consumers are often unaware that the date that is marked as *best before* indicates the time when the food is guaranteed of top quality but can afterwards be used without consequence for some time. Recognizing this food by consumers as an edible might reduce the amount of wasted food on a household level. Most respondents (85%) *agree or completely agree* with the statement that food that people could have eaten is not wasted if it was eaten by pets or composted. Although most respondents think neither of waste nor of its effects on the acceleration of climate changes (43% of respondents are neutral, and only 38% *agree or completely agree* with the contribution of food to accelerated climate change) respondents think that the population of Serbia throws a lot of food (64%), and that disorganization and laziness (79%) and consumers' fast lifestyle (67%) contributes to food waste. The majority (82%) of respondents believe that the energy, water and nutrients used in the food production and transportation are "wasted" if the bought food is not eaten. The reason for this attitude is a public misunderstanding and lack of knowledge about the problems food waste may cause such as a loss of resources used in production in the form of land, biodiversity, water, energy and inputs as well as unnecessary CO<sub>2</sub> emissions. According to a research by WRAP (Waste & Resources Action Programme), food waste emits about 18 million tons of CO<sub>2</sub> each year. WRAP estimates that if food were not discarded in this way in the UK, the level of greenhouse gas abatement would be equivalent to removing 1 in 5 cars from the road.

When asked about the safety of short-term and long-term stored food 73% of respondents said that they believe that cooked food cannot be kept in the freezer for over a year, but 53% of respondents *disagrees* and *strongly disagrees* with the statement that food kept in refrigerator for more than one day is not safe for consumption. These responses indicate that the participants are well informed that the food safety depends on longevity and type of storage of food. However, in practice this is often neglected and the food is stored longer than necessary, and ultimately thrown away, further increasing food waste. The main reason for this is



overloading the fridge with food, where food remains out of sight in storage for longer than necessary.

*Respondents' opinions in regards to wasted food.* Behaviour and attitudes of consumers before and during shopping for food have a direct impact on the way they buy food, as well as on the amount that is later wasted. Respondents were asked to choose between two completely opposite statements offered about food that describe their views best. In our sample, 61% of respondents said that before going food shopping they plan their meals and buy food on a pre-made list and during shopping they carefully think about how much food they use (89%). At the same time, in line with the current economic situation, 87% buy only food that they know it will be used, and 68% of the respondents noticed that purchased food was not used. However, the majority of respondents (90%) said they feel bad when they don't use purchased food. More than half of respondents (56%) believe that the cause of insufficient use of food was the fact that they buy larger quantities, which should last until next purchase. The reason for that behaviour was often a miscalculation of daily or weekly needs for a certain amount of food purchases, buying on sale and behaviour patterns of consumers to buy food in bulk because of price volatility, as well as the problem of continuous market supply with certain products. Among the respondents, there is increased concern for the health and safety of food so 66% throw away fruits and vegetables because they appear damaged, 63% throw food that is moldy, 55% throw fresh food passed its use date, 63% throw packaged foods that are not open, if passed use date. Although there is a view that food should not be wasted, most respondents – *answering that they still throw edible food away* - realized that throwing a small amount of food daily, expressed in monetary value, has a strong impact on their economic situation annually. A large amount of still edible food ends up in the landfill due to reasons like: health concerns, food visual appearance, various justifications in the form of being too busy, too lazy and also lack of familiarity with the "best before" issue. However, the majority of respondents (72%) show a certain kind of saving, in the context of poverty in society and the specific relationship to idea of throwing bread, which is expressed in the practice to use dry bread for toast, bread crumbs or some other culinary purposes. Respondents were asked to estimate how much uneaten food is thrown away in the household: 53% responded that they throw away very little food, 26% is throwing food away a reasonable quantity, while 18% responded that they throw more food away than is necessary, and only 3% answer they do not throw away any food. However, answering the following questions many consumers who believed they don't not throw away food came to the conclusion that they still throw a certain amount of food away, so during the survey the number of respondents who do not throw food away was reduced. The reason is the lack of recognition of food waste as an important factor in the economic sense. Respondents were asked to provide a free assessment of the average weekly amount of food bought and then thrown away unused. During the assessment of money wasted on food on a monthly basis, 50% responded that they throw food away equivalent to the amount of 1,000 dinars, 38% to 5,000 dinars, while 12% thought that they throw away food worth up to 10,000 dinars. Given that the average household income for the first half of the 2013 was 42521.00 RSD (SZS 2013), and the average basket of consumer goods was 34171.68 Dinars (Ministry of Foreign and Domestic Trade and Telecommunication 2013), this leads us to the conclusion that typical household spends relatively large amount of money on food that is uneaten and thrown away. A possible reason is that consumers, although aware of the problem, do not recognize themselves as the actors of food waste process.

*Reasons for food waste.* During our research we tried to find the reasons for consumers' behaviour in shopping, cooking, storage and food wasting. An important goal of this study was to identify areas in which consumers would show the will and desire to change behaviour when managing food in the household. Respondents were asked to identify the reasons for throwing away food at the household and to show their willingness to change behaviour for the sake of reducing food waste in the household. As two main reasons for throwing food

away in households, 21% of respondents stated that the food is thrown away because it's expired, while 14% said that the reason is they like to eat fresh food. Second group of reasons for food waste are: the food that stays long in the refrigerator and freezer as well as some members of the household do not always finish their meals 11%, 10% stated that they do not plan meals in advance, 9.5% stated that family members often change their plans during meals, while 9% don't use the remaining ingredients in other meals. From these results it can be concluded that for the majority of respondents there is no habit of planning and managing food in the household, as well as negligent practice regarding the remains of still edible food. Although many people think it is wrong to waste food, large quantities are thrown away, and are justified by being too busy and not getting the time to use leftover food from the fridge.

*Changing the planning and buying of food.* Although 97% of respondents said they are aware that they waste food and that waste as such has a negative impact on the environment and on the economic factor, only a small percentage is willing to change their behaviour when managing food in the household. In the questionnaire, respondents were offered 11 suggestions with the help of which they could intervene to reduce the amount of food waste. Of the 11 suggestions proposed 51% is *ready* or *very willing* to buy a smaller amount of food, 32% *not at all* or *not very ready* for a change, while only 14% have already implemented these changes, 51% of respondents are *very ready* and *ready* to cook the right amount of food for meals, while 33% is *not at all* or *not very ready* for such behaviour change. 49% of respondents are *very ready* and *ready* to change the way they dispose food, while 41% is *not at all* or *not very ready* to change. About 49% is *very ready* and *ready* to be more informed through information technology, and through tips from the experts. About 39% is *very ready* and *ready* to use leftovers for other meals, 45% is *not at all* or *not very ready* to change, while 31% is already using leftovers. Given that a significant part of the leftovers comes from unplanned purchase it is significant to analyze the behaviour before the purchase. Most respondents (72%) reported that they *often* or *always* check the food in the house just before buying. Based on this, 60% of respondents make a list and adhere to it as much as possible when shopping, while 37% are ready to begin using the shopping list. In the survey respondents expressed awareness that food waste monetarily valued has great economic impact on the household budget. They are also aware that better organization of purchases by planning food shopping in advance, by creating a shopping list based on their needs and weekly menu, could contribute to significant savings. However, a significant proportion of respondents are still not ready to change their old habits.

*Shopping behaviour.* Since the respondents claim that the economic situation affects the purchase, 90% of them claim to buy food *always* in accordance with the household budget. About 46% *often* and *always* buy food that is on sale, and 31% *often* and *always* buy groceries in bulk, and if the price is lower. Respondents in previous statements claimed that they have an awareness of food safety, yet only 65% said they check the expiry date before buying groceries. Only 10% of respondents recognize shopping for food in larger quantities than they need as the main reason for food waste, while 7% of respondents identify this as a secondary reason. In analyzing the reasons for the increased purchases of food, our research shows that it is mainly about the fact that our respondents believe that they really need increased amounts of food (24%), others believe that the main cause is failure to use shopping lists (11%), not checking the refrigerator before going shopping (11%) and the need to use fresh ingredients in meal preparation (8%). It is interesting that only 11% of respondents *recognize* that the reason for the increased purchases of food is unplanned buying at a discount. At the consumer level an effort to reduce food waste goes back to the purchase itself. However, consumers often fall under the influence of strong impulses such as special offers, food on sale, marketing or similar psychological traps, which are a major cause of unnecessary buying of large quantities food.

When asked what family members often do in preparing the main meal, 42% answered that they *never* or *rarely* determine the amount of servings and cook as needed. In line with that,

57% *always* or *usually* cook more than needed until the next meal. Relatively small number of respondents recognized that *cooking too much food is basic cause for throwing large amounts of food away*. In Serbia there is a lot of uneaten food left due to the cultural, traditional and hedonistic pattern of behaviour and cooking habits. That cultural pattern implies preparation of big meals in the fear that family members can't *be left hungry*, and also due to the need for variety and richness of cuisine (imperative of „good host“), as well as poor estimate of required amounts. This behaviour is typical for poor societies where *good* nutritional status is considered desirable (especially in the older generation, not burdened by trendy skinny appearance), regardless of the food quality. However, it could be claimed that due to impoverishment of society, many families in Serbia are increasingly concerned about their family budget, and thus about the amount purchased, consumed and thrown away food.

### Conclusion

Regardless of the achieved level of political and economic development and modernization of a society, lost/wasted food is important problem in economic and social terms, especially in Serbia, where the poverty rate is very high and many social categories that are barely surviving. This situation further increases the need for a scientific analysis of this issue, which at first glance may not seem so relevant, but data on the volume of wasted/lost food in some countries is alarming to address this question in the social as well as economic terms. Although there are many studies conducted worldwide, in Serbia we have no adequate analytic or synthetic estimation of total amount of wasted and lost food in various stages of the food supply chain.

This study represents one of the few analyzes of awareness, attitudes, behaviours and ways of managing food and food waste in the household and is limited to geographical area of Vrsac. However, for the purposes of this paper, only a minor share of the collected and processed data is presented, so this paper is the first in a series of papers from this empirical research that could serve to shed light on this topic and bring it to the public. In further research the authors plan a more detailed analysis of certain attitudes and behaviour patterns of respondents in the context of the types of households, financial status, level of education, the attitudes and practices, and the like.

The results of this study point to the problem of insufficient food utilization, and food waste (about 65% of respondents see it as a problem, and 68% admitted that part of the household purchased food is not used). This is mostly frozen food, food which has expired, and food leftovers due to excessive shopping, meal planning and inadequate cultural habits to prepare more food than needed, neglecting the economic value created by food waste. In short, despite the fact that there is awareness of the issue, part of the food is still thrown away. Studies of food waste is a relatively new field of domestic science with a lot of unexplored topics. This is relevant if we have in mind that empirically based knowledge about this issue could improve the awareness, attitudes and practices of waste management by consumers and institutions, but it could also be useful in the sake of creation of strategies for addressing this problem in a regional or national level.

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## OPTIMIZATION OF VEGETABLES FOR CONSUME IN FRESH CONDITION PRODUCTION STRUCTURE

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### Abstract

Vegetables production has important role in rural development. The real hypothesis for it, are: Production time of vegetables is relatively short, what give opportunity for 2-4 seeds by year; return of the capital is faster; high economic effectiveness and efficiency; vegetables is very important “healthy food”; there are a 20-30 sorts of vegetables for production in our conditions; vegetables production is very intensive.

In this paper the vegetables production structure was optimizing. Only production of vegetables for consume is optimizing. The method of linear programming is applied, by using the LINDO program. In the model for optimization, 26 of sort of vegetables are planned. Depends of vegetables position in the sowing structure, in model was included 55 independent variables, in total. For objective function, the gross margin is used (difference of total value of production, and direct variable costs (material, and external services).

The standard technologies in irrigation and actual prices of outputs and inputs are used in calculation. In the model are included next grope of constrains: agro-technical, bio-technical constrains, limitations of land capacity (first, second and third sowing), market limitations, while, limitations of manpower and machinery capacity in the months of the intensive works are not included.

The results of optimizing are showed that 41 independent variables are included in optimal structure of production. The index of soil usage is 276 %. Structure of soil usage is next: 100 % in first sowing, 98 % of second sowing, and 78 % of third sowing. Gross margin in that, optimal structure of vegetables production for consume in fresh condition is 37.500 euro per hectare of farm, per year.

**Key words:** vegetables, production, optimization

### Introduction

The main objective of this paper is to define, solve and analyze the general model for optimization sowing structure of vegetable for consume in fresh condition. On the problem of optimal vegetable production structure works Novkovic, Radojevic (2002), and Krasnic (2004), who is defined optimal models of industrial vegetables production, and vegetables for consuming fresh condition, in agricultural enterprises, and farms. Novkovic (1990, 2003), and Novkovic, Somogyi (1991) defined models for multi/criteria optimization of agricultural production.

From the aspects of using operational research methods in vegetable production, Mutavdzic et. al. (2010), and Novkovic et. al. (2009) gave contribution.

The model for optimization of vegetables for consume in fresh condition production structure, is classical model of linear programming. The Objective function is maximal economic effectiveness, as the absolute measure of economic success, expressed in money. Category which is used for express economic effectiveness is gross margin, as a difference of total

value of production, and direct variable costs (material, and external services) in vegetables production.

### Method of Research and Data Source

For optimizing of vegetables production for consume in fresh condition, based on maximal effectiveness, the classical model of linear programming is used. In the concrete model, is included 26 different kinds of vegetables. Some kind of vegetables can be included for a few times in the model, depend of sowing order (first, second, third sowing), so the total number of independent variables in concrete model is 55.

In the model is defined soil capacity on 10 hectares, and limits for second and third sowing. For that limitations the important was earlier crop (vegetable kind), and period of production, and time of sowing. That needs to define large number of agro-technical limitations. Total number of limitations in the model is 58.

The coefficient of objective function criteria is planned gross margin per hectare. Planned coefficient is accounted based on modern agro-technology of production, and actual prices of direct inputs of production, and vegetables for consume in fresh condition. Criteria function is maximal planned gross margin (value of production minus direct variable costs), what means that gross margin consist of: fix cost, indirect cost, farmers labor cost, and profit.

### Result of research

#### Definition of mathematical model of linear programming

The general model for optimizing of vegetables production for consume in fresh condition has a next structure:

#### *Independent variables:*

$$X_{ijk} > 0$$

$X_{ijk}$  – area of vegetables from group „i“, kind „j“ in sowing order „k“ in res.

$$i = 1(1)6; \quad j = 1(1)15; \text{ vegetables king} \quad k = 1(1)3;$$

i=1 root vegetables

k=1 first sowing

i=2 bulb vegetables

k=2 second sowing

i=3 tubers vegetables

k=3 third sowing

i=4 fruit-bearing vegetables

i=5 pulses vegetables

i=6 leafy vegetables

#### *Matrix of limitations:*

1. Limitation of soil capacity in the first sowing (10 hectare, or 1000 acres)
2. Limitations of soil capacity for the second sowing (limitations from 2, to 15)
3. Limitations of soil capacity for the third sowing (limitations from 16, to 26)
4. Biotechnical and agro-technical limitations – limitations of maximal and minimal areas of certain kind of vegetables (limitations from 27 to 58).

**Objective function:**

Maximizing of effectiveness (gross margin):

$$\sum_{i=1}^6 \sum_{j=1}^{15} \sum_{k=1}^2 gm_{ijk} X_{ijk} = GM_x$$

$gm_{ijk}$  = planned gross margin per acres of independent variables  $X_{ijk}$  (RSD/ )

$GM_x$  = Maximal total gross margin (RSD).

**Solution of the model**

Optimal solution, or defining of optimal sowing structure of vegetables intended for consume in fresh condition was achieved after 37 iterations, and it is presented in **table 1**. From the potential 55 independent variables in optimal solution (optimal sowing structure) is included 41.

In the model are not included limitations of direct man labor in the periods of seasonal works, because it is presumption that it is possible to provide enough good seasonal workers. Also, limitations of certain kinds of machinery are not included, too. The presumption is that, those limitations are not real limitations of production, considering the farm size (1000 acres, or 10 hectares).

In the optimal sowing structure are included: 3.99 ha (14.5%) of root vegetables, 2.51 ha (9.1%) of bulb vegetables, 1 ha (3.6%) of tubers vegetables, 1.99 ha (7.2%) of fruit-bearing vegetables, 4.77 ha (17.3%) of pulses vegetables and 13.34 ha (48.3%) of leafy vegetables.

Optimal sowing structure in sowing order (first, second, third sowing) is presented in **table 2**. Based of table data it is possible to see that for second sowing is 97% of soil and for the third sowing 78% of area of first sowing.

Maximal gross margin in optimal sowing structure of vegetables for consume in fresh conditions 42.8 millions of RSD. Converted in euro it is about 350 thousand. Calculated per hectares of farm size (10 hectare), it is 4.3 million RSD/ha, or 35 thousand euro/ha. The real indicators were obtained when calculate gross margin on hectares of used soil in all three sowing, what is 276 ha. In that case, maximal gross margin per unit of land of the farm, per year is 1.55 million RSD/ha, or 12.900 euro /ha.

**Table 1 Optimal solution of model (optimal sowing structure)**

Label i j k	Vegetables	Area ( )	Label i j k	Vegetables	Area ( )
X 1 2 2	Carrot, after kale	22	X 4 6 2	Eggplant, after peas	11
X 1 5 2	Celery, after radish	111	X 4 7 2	Pickles after green bean	11
X 1 8 1	Spring radish	133	X 4 8 2	Early cucumber, after spring lettuce	22
1 9 3	Winter radish, after early cucumber	22	X 4 9 2	Late cucumber, after spring lettuce	22
X 1 10 3	Winter radish, after eggplant	11		<b>Fruit-bearing vegetables</b>	<b>199</b>
X 1 11 2	Autumn daikon, after green bean	33	X 5 1 1	Peas	377
X 1 12 1	Early chard	67	X 5 2 1	Green bean	33

	<b>Root vegetables</b>	<b>399</b>	X 5 2 2	Turnip green bean, after early cabbage	45
X 2 1 1	New onion	22	X 5 2 3	Turnip green bean, after early cucumber	22
X 2 3 1	Spring garlic	22		<b>Pulses vegetables</b>	<b>477</b>
X 2 4 3	Winter garlic, after pepper from seed + celery	95	X 6 1 1	Spring lettuce	67
2 6 2	Leek after early chard	34	6 4 3	Autumn lettuce, after carrot	22
X 2 7 2	Winter leeks, after early cauliflower	45	X 6 5 3	Winter lettuce, after pepper from seeds & seedlings + tomato from seedlings	66
X 2 8 2	Autumn leek after early potato	33	X 6 6 1	Spring spinach	67
	<b>Bulb vegetables</b>	<b>251</b>	X 6 6 3	, after early tomato from seedlings	45
X 3 1 1	Early potato	67	X 6 7 3	Winter spinach, after carrot+ cauliflower +kale + turnip green bean	500
X 3 2 1	Potato	33	X 6 8 1	Early cabbage	45
	<b>Tubers vegetables</b>	<b>100</b>	X 6 8 2	Late cabbage, after spring garlic	11
X 4 1 2	Pepper from seed, after spring radish peppers from seed	22	X 6 9 1	Early Kale	22
X 4 2 2	Pepper from seedlings after spring garlic	22	X 6 10 2	Late kale, early potato	67
X 4 3 2	Tomato from seed, after spring lettuce	22	X 6 11 1	Early cauliflower	45
X 4 4 2	Early tomato from seedlings, after spring spinach	45	6 11 2	Late cauliflower, after green bean	366
X 4 5 2	Tomato from seedlings, after spring spinach	22	X 6 12 2	Late cabbage, after spring garlic	11
				<b>Leafy vegetables</b>	<b>1.334</b>



**Table 1 Optimal sowing structure of vegetables for consume in fresh condition, in sowing order**

Label	Vegetables	Area ( )	Label	Vegetables	Area ( )
X181	Spring radish	133	X462	Eggplant, after peas	11
X1121	Early chard	67	X472	Pickles after green bean	11
X211	New onion	22	X482	Early cucumber, after spring lettuce	22
X231	Spring garlic	22	X492	Late cucumber, after spring lettuce	22
X311	Early potato	67	X272	Winter leeks, after early cauliflower	45
X321	Potato	33	X522	Turnip green bean, after early cabbage	45
X511	Peas	377	X682	Late cabbage, after spring garlic	11
X521	Green bean	33	X1112	Autumn daikon, after green bean	33
X611	Spring lettuce	67	X6102	Late kale, early potato	67
X661	Spring spinach	67	6112	Late cauliflower, after green bean	366
X681	Early cabbage	45	X6122	Late cabbage, after spring garlic	11
X691	Early Kale	22	X282	Autumn leek after early potato	33
X6111	Early cauliflower	45		<b>Second sowing</b>	<b>977</b>
	<b>First sowing</b>	<b>1000</b>	193	Winter radish, after early cucumber	22
X122	Carrot, after kale	22	X1103	Winter radish, after eggplant	11
X152	Celery, after radish	111	X243	Winter garlic, after pepper from seed + celery	95
262	Leek after early chard	34	643	Autumn lettuce, after carrot	22
X412	Pepper from seed, after spring radish peppers from seed	22	X523	Turnip green bean, after early cucumber	22
X422	Pepper from seedlings after spring garlic	22	X653	Winter lettuce, after pepper from seeds & seedlings + tomato from seedlings	66
X432	Tomato from seed, after spring lettuce	22	X663	, after early tomato from seedlings	45
X442	Early tomato from seedlings, after spring spinach	45	X673	Winter spinach, after carrot + late cauliflower+ late kale+ turnip green bean	500
X452	Tomato from seedlings, after spring spinach	22		<b>Third sowing</b>	<b>783</b>

## Conclusion

The model for optimization production structure of vegetables for consume in fresh condition is showed the next:

- From potential 55 independent variables in first, second and third sowing in optimal sowing structure are included 41 variables from each group of vegetables: root vegetables 7, bulb vegetables 6, tubers vegetables 2, fruit-bearing vegetables 9, pulses vegetables 4, and leafy vegetables 13 variables;
- Optimal structure of production provide from ten hectares of land gross margin of 42.8 millions RSD, or 350 thousand euro.
- Optimal sowing structure of production of vegetables for consume in fresh condition provide 276 % of land usage, the most important factor of production on the farm;
- Usage of land with 276 % in intensive production, provide to farmers gross margin of 12.900 euro//ha of used land in tree sowing, per year.

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**ECONOMIC IMPORTANCE AND OF EXPORTS POSSIBILITIES YOUNG  
POTATO FROM REPUBLIC OF SERBIA**

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**Abstract**

Potato is an important agricultural cultivar that is consumed worldwide. It is a quality vegetables from which they prepare different types of food. Proteins potatoes have a high the nutritive value, and this vegetable uses as an addition to meals a with meat and dairy products, enhances the flavor, reducing energy intake and reducing the cost of food. According to the current estimates, FAO is expected that the global demand for potato in the coming decades to increase by about 35%. Potato production in Serbia meets all the needs of consumers, while the very fertile years, appear and significant market surplus that can not be absorbed by the market. Due to the limited of domestic market and saturation of demand, the development of potato production today, more needs to be based on export orientation. The problem in the realization of market surplus potatoes, may be solved only by structural changes in production, which must adapt to modern trends in the global market through increased competitiveness of supply and changing structures. In this context, market segment deals represents attractive and new potatoes. Demand for these products defines good taste, seasonal character maturities, the freshness of the product, the ability to create different specialties and so on. Good market perspective in the placement of young potatoes, can be achieved through the development of high quality of production at low costs, branding and active advertising appearance at the domestic, regional and international markets.

**Key words:** production, young potatoes, development, export

**Introduction**

Potatoes are a very important field crop in the country and has a great economic importance. The economic importance of the potato market, is determined by its use value, in the structure of vegetable and overall agricultural production, the importance of nutrition, processing and trade, foreign trade, etc. About the production and economic importance of potato farming in Serbia, can be judged on the basis of its share in the value of agricultural production. In terms of the structure of the created value of agricultural production, potatoes are in the past participated in an average of about 2.8%, while the level of production value ranged on average about 221 million dollars. Today, the potato market is becoming more segmented in terms of variety, purpose, time of arrival, etc. Production of young potato production predstavnja physiologically immature and incompletely formed tubers that are in that condition used in human nutrition. Young potatoes in Serbia for the coming harvest in the spring, when there is not a huge selection of other vegetables, and the exceptional quality of food is very appreciated, that is required on the international market. This opens perspktive for international placement home early potato.

### **Materials and methods**

The defined goal of the research will be carried out with the use of different methodological approaches. For an analysis of the production and export of potatoes used methods of descriptive statistics. Descriptive statistical analysis including methods of collection, processing and presentation of data and the methods of determining certain parameters that are relevant for the description and explanation of the changes in the observed characteristics. The main sources of data were obtained from the database of the UN Food and Agriculture Organization and the Institute of Statistics. The research is based on data available for the period 2004-2012. Relevant data were grouped and analyzed. Pimen statistical and mathematical methods are presented through tables. Applied the comparative method of analysis. We used the available literature that deals with the issue of production and export of potato and its comparative and competitive advantage.

### **Results and discussion**

Potato production in Serbia in recent decades, and has fast zna jne developmental changes, both in quantitative and structural terms. Such direction and character tendencies, followed by a permanent effort to achieve higher levels of development of this production, which caused its dynamic changes. This, among other things, reflected in the growing production, changing the traditional production-intensive, and the specific application of modern technological solutions. Despite the positive changes, potato hampered many development restrictions, which have adversely affected the country's economic position. In the current socio-economic circumstances, the basic characteristics of this production are very unsure of actual production volume of an irregular pace, unstable and underdeveloped production structures, inferior position of producers in the primary distribution which is manifested through fluctuations in product prices and price disparities, fragmentation and fracturing of possession of family farms, uncertain and unpredictable placement of the purchase price, the low level of investment to underdeveloped system of financing and lending production and processing uncertain accumulative potential, etc.

Potato producers are forced to pay particular attention to their costs of production because of market circumstances include disorganization, problems with the sales prices of potatoes, difficulties in product sales and an increase in the level of prices of inputs in production, which can cause the restrictions on establishing higher production.

In today's conditions, the production of potato were a number of non-permanent manufacturer and a small number of specialized manufacturers. It should be stressed that a number of potato reached a level of technology and competitiveness that make foreign producers in which they specialize. This group includes a number of highly specialized companies that use the latest technology. On the other hand, there are a large number of small producers who produce for their own use, the sale of the house or in the local markets. Unlike large manufacturers dominated acre specialized production, in this case, is a diversified garden and greenhouse production.

Potato producers must understand the importance of monitoring and adjusting to the needs of the modern market, both in terms of product range and volume of production and the dynamics of the arrival and delivery times and product placement. At the same time, they need to know not only the developments in the market, but long-term trends and developments as well as the legality of that act, in order to determine the proper market strategy to focus on its production. Otherwise, the business is inevitable surprises, loss or unprofitable business. Also, manufacturers should recognize the legality and the overall price

formation mechanism, demands and desires of consumers, both domestic and international markets and such needs to adapt and direct the production.

In Serbia, the average volume of actual production of potatoes has varied over the past ten years, in 2012. were produced around 578 tons. The expression of different tendencies in the movement of potato production is the result of certain economic conditions. The economic crisis has resulted in increased interest for the establishment of family farms in the production, which increased its revenue and income. At the same time, with economic development, expansion and strengthening of the internal market of agricultural and food products and increased export demand, increasingly creates conditions for increasing potato production, especially the young-early.

Potato production in the country meets all the needs of consumers, while the extremely fertile years, emerging market and a significant surplus that can not be absorbed by the market. Due to the limited domestic market and reduced demand of traditional markets, the development of potato production today, more needs to be based on export orientation. The problem in the realization of surplus potatoes, can be solved only by structural changes in production, which must adapt to modern trends in the global market by increasing the competitiveness of supply and changing structures.

Table 1. Exports of potatoes from the Republic of Serbia in terms of volume and value, 2004-2012.

Year	Total exports (tonnes)	Index 2004=100	Total exports 000 \$	Index 2004=100	The average export price \$/tonnes
2004.	9506	100	790	100	83
2005.	51235	539	2800	354	55
2006.	38529	405	2080	263	54
2007.	13576	143	2201	278	162
2008.	9961	105	1777	225	178
2009.	12578	132	1855	235	147
2010.	11423	120	2013	255	176
2011.	9321	98	3230	409	347
2012.	6116	64	1510	191	247
Ø 2004/2012	18027	-	2028	-	161

Source: FAO Database

In market conditions, the development of a sustainable and competitive production and adaptation to new consumer demands and tastes, are key factors for the development of potatoes in the need to find new approaches to markets through the promotion and diversification of production. Significant potential in this approach is the dynamic development of the young potato.

Table 2. Exports of potatoes from the Republic of Serbia by countries of destination, 2004-2012.

Year	2004.	2005.	2006.	2007.	2008.	2009.	2010.	2011.	2012.
Quantity, tonnes									
Montenegro	-	-	1063	1351	5534	4305	4913	3805	2977
Bosnia and Her	-	748	2263	5778	4329	6478	5543	625	1108
Macedonia	1711	71	204	694	25	1349	279	313	269
Croatia	4	81	197	4599	22	-	286	31	499
Romania	6801	41609	34202	-	-	23	-	-	12
Bulgaria	83	8479	421	142	-	-	-	-	-
Russian F.	63	179	59	78	31	-	305	4337	1042
Moldova	-	-	68	582	21	424	23	-	-
Albania	-	22	7	87	-	-	36	4	25
Value 000 \$									
Montenegro	-	-	237	330	1036	760	788	792	589
Bosnia and Her	38	209	342	802	702	795	900	128	270
Macedonia	145	6	23	81	4	261	73	90	51
Croatia	1	25	38	819	23	-	62	8	97
Romania	329	1939	1373	-	-	5	-	-	1
Bulgaria	5	591	32	28	-	-	-	-	-
Russian F.	7	22	19	29	10	-	177	2107	415
Moldova	-	-	6	57	2	35	3	-	-
Albania	-	4	1	17	-	-	4	1	4

Source: Statistical Office of the Republic of Serbia

Young potatoes always have a special demand and thus achieves a higher price, and on this basis, growing interest of farmers to produce potatoes before. In the areas of early production winters are mild, frost and daytime temperatures allow the unhindered development of tubers. However, it may occasionally occur nightly frosts, which can cause damage to crops. Young potatoes are usually taken out between the months of April and June, and in contrast to the traditional old potatoes, it is a fresh product that comes directly to consumers without the need for storage. Among its major market attributes, stand the taste, which is of the utmost importance for consumers. Its freshness, ease of placement on the market, the amount of sales prices, relatively little need for the use of protective chemicals and low impact on the environment during production, early production of potatoes are very attractive to manufacturers. Most of the produced young potato remains mainly in the domestic market, where it is produced, as an alternative or substitute for old potatoes from the previous year.

The economic value of this production, based on outside the of season, which allows the production of young potato and sell in foreign markets. In our country, the production is still done in small areas with a high frequency of production on small plots. Length of the cycle of production of early potatoes is the variable and it ranges from 100 to 140 days. Early potato varieties have the greatest economic impact, since grown out of season, in a cycle that is usually much earlier than the typical spring-summer cycle. Choosing the most suitable varieties is the most complex aspect of the production. Since the criteria used, the growth rate of tubers showed the greatest importance, and agricultural techniques used for growing young potato vary by region.

Based on current trends in the global market potatoes, especially noticeable growth in demand early potato. In these circumstances, new potatoes can be exported to countries in the region, which in low-yielding years have deficits in this production, but also in the Russian Federation, whose customers are more interested in local new potatoes. Exports of potatoes in

the EU, it can be competitive, because this market is close, and the cost of transport for short distances potatoes are much lower, given the fact that the potato bulky product, which suffers from low transportation costs, especially to distant markets. These processes in the market can provide a strong impetus to producers of potatoes, in establishing this production and achieve positive economic results in penetrating export markets.

For successful performance on the international market it is necessary to approach the design of optimal marketing model, determining the supply and demand forecasting, as well as creating an effective marketing mix. Young potato marketing involves delivering quality products that meet consumers' needs and achievement of profit for the manufacturer.

### Conclusion

Potato production in the Republic of Serbia has a long tradition and is characterized by rapid change and development. In the fertile years, there are significant surpluses that can not be realized in the domestic market, which implies the need for the development of potato production based on export orientation. Significant potential in this strategy, and a young potato production. Young potato is defined as a culture that is planted after winter, harvested in the spring before full maturity and sales right out. The potatoes are seasonal and fresh products of good quality, easy to use and prepare. There is a high degree of market realization, since the period of its tender is only an old potato. Our country has a favorable agro-ecological conditions for young potato, which creates good development prospects.

In the global market of potatoes, the present growth trends in demand early potato. Exports of such potatoes can be competitive and are exported to countries in the region, which in low-yielding years have deficits in this production in the EU, because this market is close to the high demand and the Russian Federation, whose customers are more interested in domestic new potatoes. This indicates that in the future there is a real market principles and a chance for local producers in the implementation of quality early potato in the international market.

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**SITUATION IN SLOVENIAN RURAL AREAS  
AND MAIN FACTORS OF THEIR ECONOMIC AND DEVELOPMENT  
PERFORMANCE**

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**Abstract**

Differences between urban and rural areas, as well as between rural areas, in Slovenia are still pretty large. Slovenian rural areas in general are highly heterogeneous, distinguished by various natural conditions and obstacles and diversified demographic, economic, and social structures. Some rural areas are more successful and more developed as others as a result of different factors. In this paper, we tried to show the differences between urban and rural municipalities and to extract factors on the basis of selected 40 indicators (demographic, economic, social, environmental) using multivariate statistical methods. The results of principal component analysis (PCA) shows that most variability between Slovenian municipalities can be explained with the factors as: productivity, entrepreneurship and investment dynamics, the structure of economic activities, economic power of the population as well as demographic factors such as population growth, educational structure, unemployment, age structure, population density etc. Influenced factors are also remoteness and state of the environment. Using cluster analysis, according to their characteristics, municipalities can be divided into four groups (so-called "typology of economic and development performance of Slovenian municipalities"). Typology can be helpful for the designing and directing of policies and measures for regional and rural development.

**Keywords:** rural areas, factors, multivariate statistical methods, municipalities, Slovenia

**Introduction**

In recent decades Slovenian Rural Areas have been exposed to many changes and challenges. Despite the existence of different development policies and programmes development differences between urban and rural areas, as well as between rural areas, in Slovenia are still pretty large. Different studies (OECD, 2006; Terluin, 2001, Bryden et al., 2004; Reimer, 2003; Perpar, 2002; Poto nik Slavi , 2008; Klemen i et al, 2008) show that some rural areas are more successful and more developed as others. Frequently asked questions of the researchers are: Why are some rural areas more successful? Is it a result of the structure of economic activities? Are these differences mainly a result of different factors like available natural resources, demographic situation, entrepreneurship tradition, infrastructure development or even a result of "less tangible" factors like partnerships, development networks, skills of local community management etc. In order to provide answers to these questions it is necessary to identify the key factors influencing the development of rural areas. To do this, first analysis of the situation in rural areas based on selected explaining indicators have to be done. Authors in their studies mainly focused on a few selected areas or regions, and on a few individual factors, while Fuller and Nichol (1999) studied the dynamics of rural economies of leading and lagging regions in Canada on the basis of a larger set of indicators that were related to economic development and the dynamics, labour force participation on the labour market, age structure, unemployment, daily movement patterns, population

structure of employment etc. They found that each region can be leading by some individual factors and lagging by the other. They noted that the assessment of economic and development performance had to include at the same time all these differing dimensions. Use of combined indicators or factors is therefore crucial, since focusing on a single indicator or factor may appear unrealistic and distorts picture of the area's situation.

### **Methodology**

Our analysis, based on a set of 40 selected indicators (explaining demographic situation, the economic performance and the labour market characteristics, quality of life and standard of living, the environment situation, the remoteness and infrastructure facilities), were carried out on administrative level of 210 Slovenian municipalities (local administrative level). For the selection of indicators it was important that the indicator helps to clarify the situation and differences in development between the municipalities (shows statistically significant differences and explains variability), and explain important factors which determines the state of economic and development performance of the area. Potential factors have been identified from the literature review and from the expert insights. The situation of Slovenian municipalities has been studied by selected indicators, as well as the differences between urban and rural communities. On the basis of different typological breakdowns we tried to determine which factors cause the observed differences, heterogeneity and variability. For the data analysis we used (beside descriptive statistics), multivariate statistical methods (principal component analysis and cluster analysis) (Johnson and Wichern, 2002).

### **Results and discussion**

The comparison of the situation between urban and rural municipalities bases on the OECD urban-rural typology for local level. The OECD methodology classifies municipalities with a population density below 150 inhabitants per km<sup>2</sup> as rural. Based on SORS<sup>41</sup> data on the population of Slovenian municipalities in 2008 and OECD criterion we identified 39 urban (18.6 % of all) and 171 rural (81.4 % of all) municipalities (Figure 1).

Figure 1: Urban and rural municipalities in Slovenia by OECD criterion.

Differences between both types of the municipalities were analysed by each individual selected indicator. Results show a high heterogeneity and variability among municipalities in general (see some examples in Figure 2).

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<sup>41</sup> Statistical Office of the Republic of Slovenia ([www.stat.si](http://www.stat.si)).

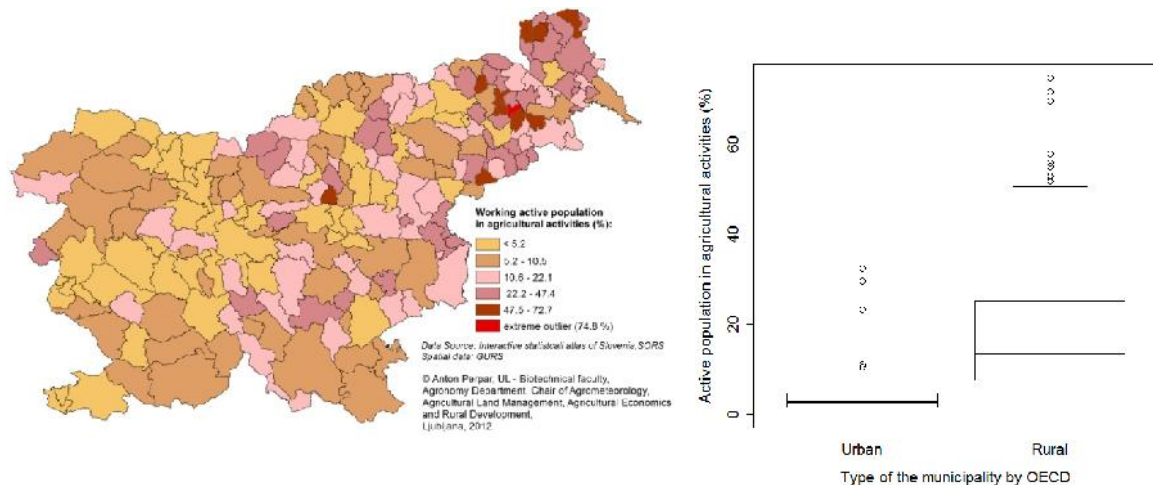


Figure 2: An example of the heterogeneity for the indicators "the share of active population in agricultural activities" (above), "the share of active population with higher education" (centre) and "registered unemployment" (below) and the differences between urban and rural municipalities (box-plot on the right of the map for each mentioned indicator).

Differences between urban and rural municipalities are the most statistically significant by population density, the share of active population in agricultural activities and the share of active population in services, the share of active population with high education, the number of registered patents and investment dynamics, value added per capita and per employee, gross taxable income, the average monthly gross salary, the number of new start-up companies etc. As expected, rural areas are in the disadvantage situation by all mentioned indicators but in more favourable situation from the environmental perspective.

Obvious differences are also between western and eastern part of Slovenia, eastern part is lagging behind, especially municipalities in the north-eastern part of Slovenia. Analysis shows that municipalities in eastern part of Slovenia have higher share of population employed in agricultural activities and opposite lower share of employments in services. Also educational structure is worst as shown by the share of active population with high education. In general, municipalities with higher share of agricultural employments have worse educational structure of population and are less developed (strong correlations - see Figure 3).

Figure 3: Some differences in employment characteristics of urban and rural municipalities (above) and scatterplots showing correlations between the "share of agricultural employment" and "development coefficient" (left) and "the share of agricultural employment" and "the share of active population with higher education" (right).

Rural areas show worst efficiency by all economic indicators (examples in Figure 4). Differences are significant particularly for value added per capita, gross investments in the

period 2000-2008 and entrepreneurship dynamics but also for innovations, business income per employee, average monthly gross salary etc.

Figure 4: Differences between urban and rural municipalities by "value added per capita" (left) and "gross investments between 2000 and 2008" (right).

From the demographic point of view population growth is still higher in urban municipalities while in rural areas we have on the one hand municipalities close to urban centres where population increase and on the other hand more remote municipalities that continue to lose population (Perpar and Udov , 2012). Negative demographic and economic trends are therefore particularly pronounced in remote rural areas (north-eastern Slovenia) and consequently cause also many problems on social field. This is faced with structural and developmental problems, which are mainly reflected in the lack of jobs outside of agriculture, higher unemployment rate, less developed infrastructure etc.

The results of principal components analysis confirmed the assumption of a large variability in the state of Slovenian municipalities. To explain a significant portion of the variability we still need several principal components. The first five principal components explain around 57 %, while eleven of them 76.5 % of the variability. Table 1 shows the indicators that importantly determinate individual principal component. Key factors, that cause variability among the municipalities, are mostly economic. The first principal component (23.2% explained variance), includes factors such as productivity, entrepreneurship, investment dynamics, the structure of economic activity, as well as demographic factors such as changes in the number of population, educational structure and socio-economic status of the population. Important factors are also the remoteness (from Ljubljana and from regional centres) and the state of the environment. The second main principal component (explains further 11.3 % of the variability) as important factors show unemployment, age structure, population density and economic size of farms. The first two principal components together are explaining 34.5% of the variability. Even in the subsequent principal components the above-mentioned factors are repeated, so we can conclude that they are a key factors of economic and development performance.

Table 1: Important indicators in principal components and factors that they are explaining.

PC	Important indicators in PC	Factor explained by indicator
PC 1	Gross taxable income The share of active population with high education Value added per employed Share of active population in agricultural activities Distance from Ljubljana The amount of municipal waste collected per capita No. of new established companies Gross investments per capita Total population growth	Socio-economic situation of population Educational structure Productivity Structure of economic activities Remoteness of the area Environmental situation Entrepreneurship Investment dynamics Population growth
PC 2	Registered unemployment rate Economic size of agricultural holdings Coefficient of ageing dependence Number of population per square kilometre	Unemployment Economic power of farms Age structure of population Population density
PC 3	Aging index	Age structure of population

	No. of registered associations per 1000 inhabitants	Engagement of civil society
PC 4	Share of active population in services	Structure of economic activities
	Average monthly gross salary	Economic power of population
PC5	Value added per employed	Productivity
	Business income per employed	Productivity
	Number of inhabitants per square kilometre	Population density

Based on their characteristics Slovenian municipalities were, using a cluster analysis, classified into four groups (so-called "typology of economic and development performance of Slovenian municipalities"). Typology can be helpful for the designing and directing of policies and measures for regional and rural development.

### Conclusions

Situation in the Slovenian municipalities is still heterogeneous. Key factors that cause diversity are economic, as proved also by the results of the method of principal components (PCA). For economic and development performance the structure of economic activities and their productivity is very important. Areas with a higher proportion of the active population employed in agriculture are economically weaker as also areas where employments in big industrial plants are predominant (nowadays mostly in troubles). Investments and innovations are very important as well. A key problem, not only in rural areas, is also unemployment, especially of young educated people, while areas with bad economic and development performance have often poor educational structure of inhabitants (due to outflow of educated people because of the lack of suitable jobs), less entrepreneurial initiative (as a result of lower attractiveness, distance to important economic centres and underdeveloped infrastructure), unfavourable demographic situation (unfavourable age structure and/or negative overall population increase). Worse economic situation of the area is reflected in social problems and the quality of life and standard of living as well. Complex mix and interactions between different factors make appropriate measures and policies difficult to design. Focus should be on improvement of regional competitiveness and activation of endogenous development potential (use of local sources, working places, improvement of infrastructure, knowledge etc.). Special attention needs to focus on improving of the business environment, and strengthening of social and human capital. In our analysis we focused on quantitative ("tangible") indicators. In practice, as well as in some other studies (as Ceccato and Persson, 2003), we can see that there are still unexplained differences in performance between areas which can be explained by "less tangible" factors such as the existence of partnerships, development networks, social capital of local communities, local leadership ability etc. They will be still a subject of future research. Endogenous development potential and entrepreneurship of rural areas are often still in latent stage, so some specific measures are needed to encourage them, as well as some technical assistance from outside.

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## STRAWBERRY PRODUCTION IN CROATIA WITH FOCUS ON VRGORAC AREA

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### Abstract

Last decade, we are witnessing increased production of strawberries in the world. In Croatia production is also constantly growing. The main Croatian areas of growing strawberries are the area of Vrgorac, Neretva river delta, surrounding of city of Zagreb, Podravina and county of Vukovar. In Vrgorac, growing strawberries for most of the producers is the semi-professional family business which presents additional family income. In Croatia are planted around 4 million seedlings on area of around 500 ha, of which 95% relates to frigo seedlings and only 5% of green seedlings. Total country production is around 3000t of strawberries yearly. Strawberries are produced on open fields and undercover in higher and smaller greenhouses. Concerning the low input costs and higher market prices it became an interesting fruit for farmers and chance to achieve higher incomes. Largest producers worldwide of strawberries are the United States of America and China. In the Europe, the largest producer is Spain. Biggest share of imported strawberries in Croatia are from Spain. Last five years strawberry production in Croatia has reached a level of self-sufficiency and achieved export potential. The main export of Croatian strawberries went the countries of European Union (EU) and Bosnia and Herzegovina. Agriculture plays an important role in country's total GDP and employment. European Union is the main trading partner, concerning that more than 60% of import and export are form the EU countries. Constraints for strawberry producers in Croatia are the level of production, uneven production techniques and land fragmentation.

**Keywords:** strawberry, Croatia, rural development, Vrgoracka jagoda

### Introduction

Strawberry (*Fragaria × ananassa* Duch.) is the most common berry fruit species in cultivation. Strawberry is also fruit with high source of vitamin C and other antioxidant compounds, such as ellagic acid and flavonoids (Robards et al., 1999). Annual production of strawberries in the world is about 6.5 million tons of fruit. In the last decade, strawberry production is increasing in the world. In Croatia we are also witnessing increased strawberry production and consumption, due to the high nutritional and health value and very early ripening (first fresh fruit market). In 2011, total production of strawberries in Croatia was 2.800 t, of which 2.000 t was for the market (CBS, 2012). Total harvested area under strawberries in Croatia is around 500 ha (author's calculation).

The main areas of growing strawberry in Croatia are the area of Vrgorac, Neretva river delta, surrounding of city of Zagreb, Podravina and county of Vukovar.

Area of Vrgorac (about 200 m above sea level) has favorable climatic conditions for growing strawberries. In that area, there are three major agricultural fields (Picture 1.); 1. field Rastok (1.675 ha), 2. field Jezero (2.914 ha) and 3. Koteze-Kokorici field (150 ha). Through that three fields pass the open surface of the river Matica which is used for irrigation of strawberry.

Population of Vrgorac (6,501 inhabitants) is situated in the twenty-four settlements and is mainly engaged in farming. Fruit growing is one of the most important branches of agriculture in area of Vrgorac. In Vrgorac, growing strawberries for most of the producers is the semi-professional family business which presents additional family income.

In Croatia are planted around 4 million seedlings on area of around 500 ha, of which 95% relates to frigo seedlings and only 5% of green seedlings. Strawberries are produced on open fields and undercover in higher and smaller greenhouses. Concerning the low input costs and higher market prices it became an interesting fruit for farmers and chance to achieve higher incomes. Largest producers worldwide of strawberries are the United States of America and China. In the Europe, the largest producer is Spain. Biggest share of imported strawberries in Croatia are from Spain. Last five years strawberry production in Croatia has reached a level of self-sufficiency and achieved export potential. The main export of Croatian strawberries went the countries of European Union and Bosnia and Herzegovina.

### **Materials and methods**

The data on the major technological and economic characteristics of production (yield, volume of production, expenses, production techniques, income and sales) of strawberries in Vrgorac were obtained by surveying 78 strawberry producers who are members of the Association of Vrgorac strawberry, for two growing seasons; 2012 and 2013. The association has 160 registered members (family farms) who are not registered for Value Added Tax (VAT).

In this study authors applied interviewing survey methods and costing (Karic, 2002). Surveys were used to collect data on production and economic characteristics of production. Applied is a form of written survey where the respondents (producers) responded to previously prepared questions about the productive resources of the farm, the quantity and value of production of strawberries and applied agro-technical operations in the production area.

This study was done within the project “Vrgorac strawberry-local product for the global market” which is financed by the Croatian Ministry of Agriculture.

### **Results and discussion**

Most of the surveyed producers (98%) have the land used for the strawberry production in their own possession, while the other (2%) are leasing the land. The total area on which analyzed farms cultivated strawberries is 24.5 ha. The average size of the family farm, which is engaged in the production of strawberries in area of Vrgorac is 3.600 m<sup>2</sup>.

In Croatia, more than 50% of small family farm where production area less than 1 ha (CBS, 2003). All of the surveyed producers are not only the strawberry producers; they are also growing other fruit species, mainly vine grapes, apples, and peaches.



Picture 1. Area of Vrgorac and fields of growing strawberry

Source: Google maps

In the area of Vrgorac technology of growing strawberries is not fully standardized (growing outdoor cultivation in greenhouses and growing in small tunnels), which significantly affects the production during ripening and fruit quality. Specifics of Vrgorac areas affecting the very early maturing and fruits appear in markets in mid-April, as the first fresh fruit and achieves a higher price. Strawberry fields in Vrgorac are highly valued and sought after due to which traders and wholesalers often marketed strawberries from other breeding areas or even from abroad called "Vrgora ka strawberry."

Producers are planting strawberries from the beginning of August till the mid of September. Due to the microlocation and climate specificities of Vrgorac area, harvesting starts at the mid April. Start and end dates of planting varies between locations (three major fields) and cultivars of strawberry that are planted.

Strawberry cultivars that are grown in Vrgorac area are: Clery, Asia, Alba, Delly, Elsanta and Madeline.

Most of the surveyed producers (95%) had high school education, 3% had University degree and 2% finished elementary school.

In both analyzed growing seasons, the soil is cultivated before planting when it is done basic fertilization or manure. Weeds in strawberry are suppressed mechanically by hilling or chemically with herbicides.

Producers starts irrigate strawberry in Vrgorac area when vegetation starts (depends of climate conditions in growing season), usually on the beginning of March.

Most of producers (76%) are selling their strawberry to the redemption center in Vrgorac, 20% of producers are selling to the local markets, while other 4% are selling them directly on their own land. Average age of respondents in the survey was 46 years, of which 83% of men and 17% of woman.

Table 1. Strawberry production in Croatia

Year	Area harvested (ha)	Production Quantity (t)	Yield (t/ha)
2011	228	2.771	12.1
2010	269	2.572	9.5
2009	257	2.548	9.9
2008	256	2.358	9.3
2007	305	3.118	10.2
2006	350	3.669	10.4
2005	500	3.221	6.4
2004	1000	4.400	4.4

Source: authors calculation and FAOSTAT

### Conclusion

In this study authors conducted field survey to collect all relevant data on strawberry production in area of Vrgorac. Obtained results differ from the official data on strawberry production in Croatia, and have to be updated. Large variation between yields is the result of different growing techniques and cultivars of strawberry. Producers are not mainly

agronomists and don't have efficient knowledge in growing strawberries. Other constraint for strawberry producers in Croatia is also land fragmentation. Our results provide baseline for further research on the market and data collection for strawberry production in Croatia.

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**AGRICULTURAL EXTENSION AND ADVISORY SERVICES IN ALGERIA AT  
CROSSROADS: PRESSING PROBLEMS AND INNOVATIVE SOLUTIONS**

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**Abstract**

Algerian farmers mostly use their own knowledge as they lack access to advisory services. Sixty-five percent of farm managers are illiterate and only 2.7% have an agricultural diploma, thus agricultural extension services play an important role in agricultural and rural development. The paper aims at analysing agricultural extension in Algeria providing a qualitative and quantitative assessment of agricultural extension services performance. The paper describes national extension strategies and action plans, analyses used participatory extension approaches and methods and explores linkages among training, research and extension components of the agricultural knowledge and information system. Secondary and primary data were used and a survey was carried out in April-June 2012 with 17 farmers in different Provinces (*e.g.* A n Defla, Batna, Chlef, Djelfa, El Oued, Gharda a, Skikda, Tiaret, Tipasa). A SWOT analysis of the agricultural sector was performed. Most of the interviewed farmers consider information and advice provided by public extension agents very useful. Nevertheless, producers rely also on other information sources such as other farmers and the private sector. The lack of an enabling institutional, legal and political environment - especially the lack of a national extension strategy - is an important gap. Collaboration between researchers and extension agents as well as the involvement of farmers in the development, validation and dissemination of technical and social innovations is highly recommended. Information dissemination and communication based on the interaction between formal and informal networks may be interesting in the current context to meet farmers' needs.

**Keywords:** Agricultural extension, Institutional and political environment, Algeria

**Introduction**

In Algeria, the utilised agricultural area (UAA) is about 8.458 million ha, representing 20.8% of the total agricultural area (TAA) and only 3.54% of the total land area (TLA). There is a total of 1,023,799 farms. Small farms (0.1 to 10 ha) account for 70%, covering 25.4% of the UAA (MADR, 2008).

Today, agriculture employs about 26% of the total population and accounts for 8.6% of the gross domestic product (GDP). The low productivity makes food import necessary, only 25% of food needs are met by domestic production (Belabas, 2010). The agricultural trade balance deficit is regular. Nevertheless, efforts are undertaken to improve the agricultural sector performance. The main crops are cereals, including wheat and barley. Other important agricultural products include potatoes, grapes, citrus, olives and dates (Medagri, 2006). In general, crop productivity is weak compared with neighbouring Morocco, whereas the yield is

at least double overseas. Other major problems are related to agro-food products quality (Bedrani, 2002).

Several factors have hampered development in Algeria, including land ownership constraints, lack of investment, insufficient access to input, reduced water availability, low levels of education and agricultural training, lack of extension services support, marketing channel constraints and bureaucracy (Laoubi and Yamao, 2012).

In terms of training, 65% of farm managers are not educated, 1% have a high level of education and only 2.7% have an agricultural training. Almost a third (32.6%) of farm managers under 40 are uneducated; this rate is much higher among heads of holdings aged over 60 (Rebouha, 2005).

Agricultural extension and advisory services play an important role in agricultural development and can contribute to improving the welfare of farmers and other people living in rural areas (International Initiative for Impact Evaluation, 2010). Agricultural information is a determinant of farmers' adoption of new farming practice and agricultural technology, and thus, achieving agricultural development goal (Laoubi *et al.*, 2010).

One can simply say that “*extension is getting knowledge to farmers so that they will make a positive change*” (USAID, 2012). Advisory service is commonly used as an alternate term for extension services. Apart from their conventional function of providing knowledge and technology to improve agricultural productivity, agricultural advisory services are also expected to link farmers to markets, promote sustainable production techniques, etc. (Swanson and Rajalahti, 2010). Good extension is recognized as a key to agricultural development. For extension to be successful, it needs to include credible content, effective delivery and be relevant to and applicable by clients (USAID, 2012).

Multifunctionality in agriculture and rural economy diversification are changing dramatically the classical crop production-centred mission of agricultural extension and advisory services (World Bank, 2008). Farmers in Algeria have a number of information and support needs to enable them to operate in competitive environments that exceed the traditional requirements for agricultural technologies. These new needs pose new challenges for both research and extension services (IFPRI, 2012).

The main objective of the present paper is to analyse Algerian agricultural extension system setup, governance and performance.

### **Material and methods**

Secondary information from different sources (*e.g.* IFPRI, GFRAS, MARD) as well as primary data were used. A SWOT analysis of the agricultural extension sector was performed. A field survey was carried out in April-June 2012 with 17 farmers dealing with crop production (tomato, citrus, pome fruit, potato, cereals, corn, garlic, etc.) and animal husbandry (*e.g.* camels). The survey was carried out in collaboration with the staff of the Ministry of Agriculture and Rural Development (MADR) especially the Directorate of Agricultural Services (DSA). The visited areas are located in different regions and provinces (Wilayas) of the country: coastal region (Tipaza, Chlef, Ain Defla), central region (Djelfa, Khemis Miliana), eastern region (Batna), western region (Tiaret and Mostaganem), south-eastern region (Skikda) and southern region (El Oued, Ghardaia).

The fieldwork was designed to allow the collection of information on planning and management of local extension activities; the system of agricultural knowledge and new information and communication technologies used in current extension activities; methods and effectiveness of extension applied; and the level of satisfaction of farmers of the extension system. In this context, a checklist was prepared to collect basic data on the profile of farmers and their farms and their views about the extension system.

## Results and discussion

According to Benfrid (1997), agricultural extension in Algeria is often reduced to the decomposition of the technical package, a set of topics to raise the awareness of the farmer. Farmer are considered as passive receptors.

In Algeria, since 1985, several actions and measures have been undertaken regarding agricultural extension. A ministerial Circular No. 1065 of 31/12/1985 has set up an extension system and organizational structure integrating all stakeholders. The executive Decree No. 95-99 of April 1<sup>st</sup> 1995 established a National Institute for Extension (INVA). The decree No. 96-97 of April 13 1996 provided status recognition for extension agents and as consequence devoted the extension as an important function in agricultural development (Laoubi *et al.*, 2010).

The Worldwide Extension Study (WWES) responses - received from each of Algeria's 27 district offices of the MADR – showed that Algeria has a total of 834 public extension staff of which 722 are field-level extension staff. About 82% of the field-level extension workers are male, with 65% of them holding a 2-3 year agricultural diploma. Most of the women extension workers are field-level (130 women staff) with 46% of them having a MSc/agricultural engineer degree (IFPRI, 2012).

The MADR delegates extension to the Directorate of Agricultural Services. Within the institutional framework for extension the primary responsibility for program planning falls at the local and national levels and the primary linkages are agricultural schools and input supply firms. Farmer organizations have a role in helping set extension priorities and promoting farmer-to-farmer extension activities (IFPRI, 2012).

The extension system is linked to the economic, social and cultural context in which it operates and that affects the quality and effectiveness of its actions. The current organization of the extension system in Algeria is reported in table 1.

**Table 1.** Organization of the agricultural extension system

Stakeholder's functions	Main stakeholders
<b>Administrative</b>	Ministry of Agriculture (Sub-directorate of Extension, central technical departments); extension committees of provinces ( <i>i.e.</i> Wilayas); offices of education and extension (Directorates of agricultural services); Section of agriculture of communal agricultural delegations
<b>Technical and scientific</b>	National Institutes (02) of agricultural and forestry research (INRA and INRF); Specialized/sectoral technical institutes (07); institutes for plant protection (01) and animal medicine (01); High commissions for steppe (01) and Sahara zones development (01)
<b>Technical and logistics</b>	National and Regional Offices; Cooperative Service; Union of Cooperatives; National Fund of Agriculture; Bank for Rural Development; inter-professional councils; economic enterprises and services; management and participation companies (SGP)
<b>Methodological support</b>	INVA; agricultural training institutes; Coordination Committee of CCRAFAT (Agriculture and Forestry Research and Technical Assistance)
<b>Agricultural profession</b>	National Chamber of Agriculture and chambers of wilayas (47); agricultural professional associations and NGOs; Agricultural Syndicate (National Union of Algerian Peasants)
<b>Support structures</b>	Mass Media (Radio, TV and newspapers)

The primary extension activity is educational and advisory and the principal institutions are agricultural schools (IFPRI, 2012). The Global Forum for Rural Advisory Services (GFRAS), in its Directory of Extension Providers, identifies 13 public extension providers in Algeria (GFRAS, 2013). The main institutes and research centres that have a stake in agricultural extension in Algeria are (Korichi, 2010): National Institute of Agronomical Research of

Algeria (INRAA); Technical Institute of Field Crops (cereals/forages) (ITGC); Technical Institute of Industrial Crops and Gardening (ITCMI); National Centre of Control and seeds and seedlings Certification (CNCC); National Institute of Plant Protection (INPV); Technical Institute of Livestock (ITELV); National Institute of Orchards and Vineyards (ITAFV); National Institute of Soil, Irrigation and Drainage (INSID); National Institute of Forest Research (INRF); National Centre of Artificial Insemination and Genetic Breeding (CNIAAG). The High Commissariat for the Steppe Development (HCDS) and Commissariat for the agricultural Development of Saharan Regions (CDARS) are active in marginal and less favoured areas (Korichi, 2010). Small-scale subsistence farmers and large commercial farmers are the clientele most targeted by extension activities (IFPRI, 2012).

However, a crucial role in the agricultural extension system is played by the National Institute of Agricultural Extension (INVA). Its missions are (Korichi, 2010): studies and investigations (systems, approaches and extension methodology; communication channels, means and techniques); technical support activities (coordination and facilitation mechanisms; development of extension campaigns and programs; monitoring and evaluation of extension activities; development and evaluation of training programs and capacity building cycles); and production and dissemination of extension materials.

National agricultural extension services through their regional stations located in all the provinces (*i.e.* Wilayas) of the country implement various extension activities for farmers on different topics according to crop and livestock species that characterize each region. Extension at province level (*e.g.* Blida) is ensured by the collaboration of various agricultural institutions, such as DSA, agricultural subdivision, chamber of agriculture, and technical institutes (Laoubi *et al.*, 2010).

The extension strategy and operational plan for the five-year period (2010-2014) focuses on participatory planning, intensive training of extension workers, development of multi-media materials, and processes for managing, monitoring and evaluating actions. These activities are intended to be applied by extension staff to support the policies, strategies and priority programs of agricultural and rural development.

The primary extension method is through demos, workshops and field days followed by farmer field visits. Radio programs are the main means of ICT (IFPRI, 2012). The Ministry of Agriculture has added a network of demonstration units preferentially located at farmer level and in the 69 stations of the centres and institutions disseminated in Algeria. Agricultural information and dissemination methods in place are numerous (Korichi, 2010): traditional ones, using the support of papers/newsletters, scientific/technical and extension meetings, conferences and workshops; and some are using the ICTs, such as websites ([www.inraa.dz](http://www.inraa.dz), [www.dgrsd-dz.org](http://www.dgrsd-dz.org), [www.anvredet.org.dz](http://www.anvredet.org.dz)).

The analysis of the results of the survey provides several insights on the performance of the agricultural extension system in Algeria. Farmers often live in total isolation from the institutions of the state in terms of agricultural finance, activities and projects. More than a half of the farmers strongly affirm that the information made available by the extension services is a way of knowing other agricultural techniques, which confers the extension work a more innovation-oriented than a problem-solving feature. Just over 10% of farmers said that the information available to them is unnecessary. Data from this survey highlight the importance given to information exchange in the farming community. There is a system of sharing agricultural knowledge from different sources: other farmers, extension agents, private sector companies, etc.

Even though, it can be noticed the importance of informal information networks, about a third of the farmers relies on extension agents and a quarter relies on private companies as a means of access to knowledge.

If we refine the analysis of information transfer by technical institutes and main areas of activity, we realize that information on field crops is ranked at the top with an average of 42.7% (41% for cereals and 44.4% for fodder). By cons, less than a third of farmers reported having been informed in the areas of arboriculture, horticulture and livestock.

Farmers are still using their own knowledge, or advised by unqualified persons as agricultural inputs sellers or some state employees who are not educated enough. Even efforts made by several technical institutions are not sufficient because of the complexity of problems and the ineffectiveness of their extension methods, which are of top-down nature. Farmers need to be assisted by extension organisms or individuals who know their real difficulties and know their practical situations to overcome their problems. Extension services have to be effective and sustainable. Improving the capacity of individual and collective producers allows short-term facilitation, a better match between technical solutions and the constraints faced by the farmers, and in the long term, fostering their constant search for innovation.

The very high percentage of uneducated heads of farms in Algeria affects negatively the effectiveness of the national extension system. However, the SWOT analysis prepared with rural people and farmers (Table 2), shows that we can be optimistic because of the emergence of a new generation of well-educated farmers who are very motivated to improve and modernise their activities. The most important gaps are still the lack of regulations, rules and a national strategy regulating extension domain. The state has also to increase the number of extension agents to meet the whole farmers' needs in terms of information and advice.

**Table 2.** SWOT analysis of agricultural extension system.

<u>Strengths</u>	<u>Weaknesses</u>
<ul style="list-style-type: none"> <li>• Support of farmers by professional organizations and institutions</li> <li>• Establishment of a training program to young farmers</li> <li>• Emergence of modern farms very interested in extension programs and training</li> <li>• A new generation of young farmers, motivated and innovative</li> </ul>	<ul style="list-style-type: none"> <li>• Weak affiliation of farmers to professional associations and unions</li> <li>• Lack of a communication policy</li> <li>• Mismatching between the information needs of farmers and supply</li> <li>• Difficult access to agricultural information and knowledge</li> <li>• Aging of farm managers</li> <li>• Qualified staff is insufficient and unevenly distributed</li> <li>• Lack of financial and material resources</li> <li>• Lack of tools for information retrieval</li> <li>• Very low number of extension materials circulated</li> <li>• Slowness of responses to information requests from farmers</li> </ul>
<u>Opportunities</u>	<u>Threats</u>
<ul style="list-style-type: none"> <li>• High motivation of new and young farmers to improve their knowledge</li> <li>• High number of underexploited agricultural extension and research infrastructures (ITMA, CFPA...)</li> <li>• highly qualified human resources (agronomists, biologists...) to implement extension projects</li> </ul>	<ul style="list-style-type: none"> <li>• Lack of regulation and rules for extension</li> <li>• Practical problems hampering agriculture and rural development</li> <li>• Lack of effective extension linkages between the agricultural sector and research</li> <li>• Loss of trust between rural stakeholders and advisory bodies</li> </ul>

## Conclusion

Algeria is willing to support farmers, faced with a very complex situation. It developed an important support system including measures to promote professional organizations and institutions and to support agricultural production. However, farmers do not have the feeling of being supported by them effectively. The extension system is ill-performing and focused on crop production especially field crops (cereals).

The use of modern means of communication can diversify the sources of information of the farmer and ensure better coordination between the actors involved in agricultural production. It also allows proposing an information offer diversified and adapted to the different categories of farmers. New approaches to ICT for agricultural development and natural resource management exist to overcome extension challenges and new concepts are emerging for participation, learning and problem solving between the key players.

Today, Algerian farmers are increasingly asked to respond to the challenges facing them. The profound changes occurring in the agricultural environment also require them to have more knowledge in agronomy and related sciences, management, accounting, marketing. The availability of extension agents and the ability to exchange and validate new techniques and/or productions to introduce at the farm level by researchers and specialists, are highly desired.

Due of its status, the national system of agricultural extension led by INVA could probably be an answer to the needs of pooling local resources and opening to international networks. This is related to the need to restructure the system, from the organizational and functional points of view, and to strengthen its human resources by mobilizing sectoral competences and external resources. Analysis of informational needs of farmers should constitute the basis for building the extension offer.

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**THE ROLE OF WOMEN IN EXTENSION EDUCATION AND RURAL  
DEVELOPMENT IN FINLAND**

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**Abstract**

This paper is to find a larger meaning of Extension whose aim is to disseminate the advisory work to usual consumers. Food security is not anymore the task of agriculture alone. The small farmers and the part time farmers are more responsible of food security. Moreover, the industrialized farming has changed the role of extension.

The small farming needs the local advisory work. The women are not mainly responsible of the agriculture and the farming in Finland. Food security and food safety is generally organized by women associations. In Finland the voluntary based women organizations take care of disseminating the information of food security to the consumer level. There are not very small family farms in Finland. The food is produced by the bigger farms. There are less and less farms in Finland; at the moment about 59 000.

The role of women has risen almost dramatically in the food chain. The voluntary based women's organizations have become more important in education about food safety. The obesity is not that severe problem but to disseminate innovations of the healthy food is on the program of extension education.

The ProAgria is called the Finnish national advisory work organization. The Country Women association, Maa-ja kotitalousnaiset (45 000 members) is part of ProAgria. The other important voluntary based groups of women for the extension education are the Martta's (60 000 members) and Swedish speaking Martha's (20 000 members). The Extension as such is not educated or researched at the university in Finland. The women as students in the colleges have become more key persons in food security.

**Keywords:** extension, gender, women, advisory services.

**Introduction**

The Extension education is a term for advisory work. The Extension started at the universities in USA after the Second World War (Alford, 1968). The need of information of the modern agriculture was important. In Europe the war has destroyed the agricultural professional knowledge. The famine was every day; the fields had been used poorly for food production during the war.

The Universities in USA had continued the agricultural research. The students from Europe visited and studied to learn the modern agriculture and the new tools to produce food. That is the reason for using the American word "Extension" globally.

Extension is used for general dissemination processes for policy and research information which is considered by its producers to be relevant and useful for farmers. The other words which are used to Extension are advice and consultancy.

*"Advice will mean the definition and clarification of problems and the options for their solution, arrived at through a personal relationship between an advisor and consumer. Consultancy takes advice further to arrive at the choice of specific proposed course of action with the justifying arguments and evidence."* (Rolls, 2000).

The role of universities is assumed to include that of proposing general explanations of the main process of information transfer in agriculture. This information is based on research, innovations and analysis, as well as assisting the development of extension systems contributing to them.

In this paper Home Economics (HE) will be used according to the IFHE Position Statement in the 21<sup>st</sup> Century (IFHE, 2008) as “*an arena for everyday living in households, families and communities for developing human growth potential and human necessities or basic needs to be met*”. The global and local contexts will be taken together into consideration in this paper.

### **Meaning of Extension within Home Economics**

Traditionally extension has been a task of agriculture and farmers. This paper has larger meaning to the consumers than to producers of food. Food security is important and not only about agriculture. The small farmers and the part time farmers are more and more responsible of food security beside the big farms. The industrialized farming has changed the role of extension.

The small farming needs the local advisory work. The women are more responsible of small farming. It means that food security and specially food safety belong to the women.

There are not very small farms in Finland. The average size of farms has grown during last 20 years 70% from 22.8 ha of arable land to 38.6 ha. The reason is that the total number of farms (over 1 ha) has fallen to 58,900. This was 2,300 farms (3,7%) less during one year 2011-2012. During seventeen years in the EU the number of farms in Finland has fallen 38% from the 95,500 farms to about 36,664 farms. While the number of farms has fallen the average size of farms has been growing. Finnish agriculture is almost exclusively based on family farms. 87.9 % of farms were privately owned in 2012, and the 10.7% were owned by heirs and family companies and corporations. Some percentage (2%) were owned by cooperatives, limited partnerships and the State, municipalities, schools and parishes.

The average age of farmers on farms receiving agricultural support was 51.7 years. Since 1995 the average age of farmers has risen by about three years. As the farm population is ageing, the share of young farmers is falling and that of the older ones is growing. In 2001 the share of the farmers over 55 years of age was 26%, but in 2012 their share was as high as 39%. During the same time the share of the under 44-year-old farmers fell from 38% to 28% (MTT, 2013).

### **Production structure of farms in Finland**

Measured by the number of farms the production structure of Finnish agriculture has changed considerably in recent years. The utilized agricultural area in Finland is about 2.3 million ha, which is 6.8% of the total surface area and 7.5% of the land area. Compared to the average in European Union, the share of agricultural area is very small in Finland.

The share of livestock farms has fallen while the share of crop farms has increased clearly. In 2012, 27% of the farms which applied for support were livestock farms and 67% were crop farms while in 1995 the share of livestock farms was 52% and that of cereal crop farms was 39%. In 2012 cereals were cultivated on about 1,036,000 ha in Finland. The total cereal crop was about 3,668 million kg.

**The forest** is an integral part of Finnish farms. In 2012 the average forest area of farms was 50.7 ha. Regional variation is considerable. In Southwest Finland the average forest area of farms is 31 ha while in Lapland it is 108 ha.

### **The food market**

The domestic food market depends on import (MTT, 2013). The foodstuffs that would be fully domestic existed only in the past. Today's agriculture and food production cannot be successful without imported inputs. In reality purely domestic agriculture may be considered to have ended when tractors substituted for the work done by horses. A lot of the human labor in the various tasks of the production chain has also been replaced by machinery and devices which depend on energy from foreign sources.

### **The consumer prices**

In 2012 the food prices in Finland rose by 5.2% from the year before (MTT, 2013). The annual consumer price index was 2.8% which means that the rise in food prices was more rapid than the general inflation trend.

### **The meaning of Extension with Home economics**

This paper is to find a larger meaning of extension. Food security is not only the concern of agriculture any more. The small farmers and the part time farmers have become more responsible of food security. In addition, the industrialized farming has changed the role of extension. The whole picture of food production has changed

The small farming needs the local advisory work. The women are not traditionally responsible of the gardening. The food security belongs to the women in general. Home economists study nutrition. Nutrition deals with foodstuffs that come from the fields and gardens to the table. Home economics (HE) is taught at universities and colleges in Finland. The extension education as such is not taught at the university level in Finland. That is why the role of home economics has become more important to the extension and advisory services research.

### **History of home economics teaching in Finland**

Education to girls was allowed in early 1800 in Finland. The home economics teaching started in 1870 in order to teach the poor girls to prepare good food and clean houses to the 'better' people (Laine 1931, Levanto 2013). The specified teaching of home economics was not common of long period. In 1980' it was normal to have home economics and house hold economics education in the schools, since 1963 to boys as well.

The school reform in 1970's finished almost all home economics teaching in schools.

In 2012 there is again a fighting for a new structure of curricula to get more practical issues to school programs. The home economics teachers, women organizations, parliament members, individual people have signed petitions to get in the school curriculum more lessons a week and during school time altogether dedicated to the home economics, handicraft, gardening, nutrition, consumer sciences.

### **The voluntary based home economics organizations**

Late 1800 started a very active movement for civilization of women in Finland.

The politically oriented and educated women opened the discussion about the rights of women to take care of their life and the family life (Laine 194; Heinonen 1998). Worldwide there was the same emancipation movement.

The Martta organization was established in 1892 to teach "Civilization to women". It was taken a short name according to the bible, Martta and Martha's. The Country women organization was first a part of men's extension organization. In 1920-1930 it found a way to be independent part of Country Women. Now it is called Country and Home Economics Women.

Country women have always been more land use professional orientated than Martta's or Martha's. The Country Women have been farmers' wives, small or big farms but having land

to cultivate, and animals to produce milk, wool, meat. That way it was possible to develop many important products for human life in society.

All main women's organizations have had international contacts. Country women are members in ACWW (Association of Country Women in the World).

Martta's are very active internationally during time of extension and home educationist, MSc Maija Riihijärvi-Samuel, started the extension education at the University of Helsinki in 1970. Later she was a managing director in Martta organization.

Martta continues as a member of IFHE, the International Federation of Home Economics.

The statement of 2008 gives the structure for the IFHE program and meaning of Home Economics.

Specially Country women had very active 'surviving courses' during second world war.

The Martta's has more difficult task without land ownership.

### **The education of Home Economics and Extension at the University**

The model and the example of the American universities prepared the atmosphere so that in the 1946 the Department of Household Economics was founded at Helsinki University situating in Agro-Forest Faculty. In that decade there were several schools of home economics, cookery and gardening training schools, and home economics advisory organisations as well as two agricultural home economics polytechnics and colleges.

The education of food production belongs to the home economics department and faculty of education at the universities, now. The household economics is in program at the University of Helsinki. As there is no research or education of extension at the university in Finland, voluntary based advisory organizations have important role of rural development. The ProAgria is a private extension education information and advisory work organization. The Country Women, 'Maa-ja kotitalousnaiset' is a female part of ProAgria with its 60 000 members. The other voluntary women's advisory groups are Martta, 45 000 members, and the Swedish speaking Martha, 20 000 members.

### **Research organizations**

Työtehoseura Work Efficiency Institute (TTS), has been since 1946 very active research organization in agriculture, forestry and home economics. TTS organized the big exhibitions and participated in all the exhibitions concerning agriculture, forest and home tools new innovations until recent times.

Agro-nutritional Research Center (MTT) is disseminating innovations via publications and books. A lot of seminars have been organized to introduce new innovations as well as seminars for rural development.

### **Historical transformations of Rural Finland**

The area that nowadays represents Finland has been populated for very long time, several thousands of years. In effect, at the beginning of our chronology there was a growing stream of immigration over the Gulf of Finland, encouraged by the good prospects for hunting and trapping fur animals in the immeasurable Finnish woodlands. That export of furs united Finland economically with Europe for the first time during the first centuries of our calendar (Katajamaki 1999, p 11). We can imagine that the same kind of economic immigration continues now i.e. employees immigration from Estonia to Finland. The Finnish firms give work in Estonian companies and Estonians people come to work in Finland. The same kind of exchange was 40 years ago from Finland to Sweden, and one hundred years ago to USA.

Due to abrupt changes in the countryside, farmers founded an occupational organization, the Central Union of Agricultural Producers (MTK) in the beginning last century.

**The rural Finland** is working hard for agriculture and food security now. Agriculture is not fashionable as profession. The environmental issues and climate change are on social media. The local food is important, the home and city gardening are necessary. The education of small scale food production has found a way to teach the immigrants their food and further on their families fire wood and constructing trees logs. The farmers in their real own farms work too much. The farmer family must work outside farm to survive. The government makes the constructing in rural areas almost impossible. The new buildings are refused in the rural areas. The saving in community level is ridiculous. The farmers find it difficult to renovate the farm buildings. There are new people coming to the rural areas but the new constructing is refused (Sarvijarvi, 2013).

### Conclusion

Extension education is presented as an important part of home economics education. Home economics is in close contact to the agriculture and that way to food security. Home economics have taken the task of disseminating the new innovations of everyday life. Voluntary based organizations have got more important role to make the extension of innovations to usual consumers. The ProAgraria organization is active in agricultural innovations and disseminates all novelties to rural professionals. The Forest Center Metsäkeskus and Forest Company Metsänhoitoyhdistys are active in forestry extension work. The extension research at university level as such is looking for a better future. The voluntary based and hardworking ‘extensionists’ need national research. International education is well known but the local and national studies at university level cannot be substituted.

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## THE IMPACT OF SERBIAN AGRICULTURE EXPORTS ON ITS TRADE DEFICIT

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### Abstract

Exports of agricultural and food products is the consequence of high and efficient domestic production and favorable agro-ecological conditions, but also the proper implementation of agricultural and food policy. There are significant restrictions on trade in agricultural and food products. In recent decades the agricultural protectionism comes to the fore; that has emerged for the protection of agricultural production in developed countries from the extreme competition in the international market. Strong technological progress in many countries encouraged agriculture production that has resulted with the surplus in agricultural products, after long period of recorded deficit. At the same time, exporting countries are trying to increase productivity in order to retain their existing positions in the international market. For these reasons, there is the instability of the world market of agricultural and food products, which particularly affects the economically undeveloped countries.

Serbian agriculture is gradually losing its leading position in the region. From the fact that the agriculture and food industry, with still unused possible resources, have significant impact on the reduction of the current account deficit and because of the necessary adjustments to future European integration, it is expected that they, therefore, have better support from the government. Unfortunately, this is not the case, and as a consequence of this attitude of the state towards agriculture, it achieves significantly lower results in the export than possible. Agriculture budget should be development oriented, to the improvement of agriculture and increase agricultural exports, and not be so often misused as a purely social category.

**Key words:** agriculture policy, Serbia, trade deficit, exports

### Introduction

Modern economies are more or less open to the world market. The degree of openness varies from one country to another. The rate of economic openness (the share of exports and imports in Gross Domestic Product-GDP) is very different. Public finance policy, foreign trade policy and development policy are interdependent segments of macroeconomic policy (Johannes & Wetzel, 1988). Interaction is established through the real exchange rate, foreign exchange reserves management, external debt, the budget deficit management and the policy of liberalization of foreign trade. Contemporary countries react quite differently to external shocks. Countries with a relatively stable exchange rate, with rational tax operations (tax pressure) and the liberalization of foreign trade have more easily overcome the economic crisis and relatively quickly stimulated economic activity and development. Any mismanagement of fiscal deficit is often the cause of overvalued exchange rates and all that because the deficit is the additional requirement of an established government offer of goods in the economy, which is purely inflationary method (Stiglitz, 2002). By increasing the budget deficit the state is putting further pressure on domestic demand, which leads to an increase in



prices, interest rates and wages. Each oversized reclining on the creation of base money to finance public deficit, further encourages the growth of domestic products' prices, which compared to the prices of foreign goods, creates the overvaluation of the real exchange rate. The overvaluation of the exchange rate, on the other hand now, favors the production for the domestic market, thereby drastically affects the loss of competitiveness in international markets. Therefore, export is limited, import is increased and all that worsens country's balance of payments position. In modern, industrialized countries, public expenditure policy (level and composition of public spending) is an important determinant of economic growth and foreign trade. Public expenditures of the state through public investments influence economic development, and consequently, the foreign trade (Ram, 1986). Public investment in the agricultural sector, road, water, and energy infrastructure, research and innovation services are reasonable examples of supporting the production of goods for foreign trade. Efficient allocation of public expenditure can ensure the increase in production intended for foreign trade, in order to mitigate impacts of external shocks and to encourage private investment in the changing of economic structure and diversification of export activities.

Agriculture and food industry of Serbia is the backbone of economic activity and is the only area beyond the defense industry, which generates a surplus in foreign trade. Agricultural and food sector in Serbia are export-oriented and in 2011 the total trade exchange in these sectors amounted to about 4 billion dollars. Total exports of agri-food products to the world market amounted to 2.7 billion dollars in the same period. All other economic sectors recorded trade deficit and raised the debt level. From the fact that the agriculture and food industry, beside the food security of the country and the impact on the reduction of the current account deficit with foreign countries, have untapped resources and because of the necessary adjustments to future European integration, it is expected that they, therefore, have better support from the government. Unfortunately, this is not the case, and as a consequence of this attitude of the state towards agriculture, it achieves significantly lower results in the export than possible.

Serbian trade deficit has grown considerably in recent years, so that in 2009 was close to 18 % of GDP, which is one of the largest deficits in the region. What is disturbing, however, is the fact that the growing external deficit is not a result of the expansion of private sector investment that could pay off in an improved supply and export growth, but is outcome of high level of consumption. Long-term overrated value of the dinar was stimulating imports, increasing the trade deficit and external debt, so that the achievement of sustainable relations with other countries (as measured by the balance of payments) was unsatisfactory. So, it is understandable why there are fears regarding macroeconomic stability of the country in the coming years unless export is increased and rapidly borrowing as well as domestic consumption are stopped.

### **Materials and methods**

In order to analyze properly the issue of increasing exports of agriculture as one of the necessary prerequisite for the stabilization of the Serbian economy, the role of the state as a key factor in terms of funding and subsidies to agriculture, secondary sources of information that provide relevant statistical data, such as the World Bank, Serbian Chamber of Commerce, Statistical Office of the Republic of Serbia, were used. The survey is also based on professional literature, that contains the facts about the topic, as well as other types of publications, which in some way discuss the above issues. Methodological principles and tools that are adopted in the work are the statistical methods, SWOT analysis, induction and deduction, as well as the technique of comparative analysis.

## Results and discussion

Serbian agriculture is gradually losing its leading position in the region as evidenced by the fact that exports per hectare of arable land is less than double than the agriculture export of Macedonia (FYROM) and even more than the agricultural export of Croatia. Measured by the export per hectare, Serbia is still better than Romania and Bosnia and Herzegovina, which are working very hard to change this situation. The transition of agriculture to market conditions is accompanied by significant development of foreign trade. While in the 90s and in the first half of 21<sup>st</sup> century there was permanent trade deficit (except for a small surplus in 2000), from 2008 to 2012 a significant surplus has been recorded. However, this information should not be accepted as a fact that there has been some kind of improvement in the agricultural sector, but it is due to the bilateral agreements with countries in the region (Central European Free Trade Agreement - CEFTA), the transformation of the Montenegro market into a foreign one, the free trade agreement with Russia and quotas for the preferential export to the European Union (EU).

Today's state of Serbian agriculture, its potentials and limitations, still have the same trend - that in agriculture are less inputs than are really needed, and therefore the results are lower than desired. In the process of planning and directing state aid has not been established yet a system of coordination between many institutions and actors involved in all aspects of rural development. The issue of state subsidies, precisely to whom should be given and to whom not, always brings strong reactions by the public. Agriculture budget should be development oriented, to the improvement of agriculture and increase agricultural exports, and not be so often misused as a purely social category. It is enough to look for appropriate arguments to support this claim with the practice of countries where the agriculture is far more developed than in Serbia, and even in neighboring countries. Their leading producers receive enormous subsidies that could be millions of euros, what place agrarian systems in Serbia in an inferior position, when competing in the international market. These countries do not insist at all costs on liberalism. Agricultural policy, which gives priority to safe, quality food, and at the same time takes care of environmental protection, is the only acceptable policy. In order to achieve this, significant financial funds are allocated to subsidize agricultural production. But the essence is in more effective redistribution of funds from the general budget and in much better allocation of resources. Thanks to such a way of running the agricultural policy, in market oriented economies, a sufficient quantity of food is provided to cover their own needs and for export as well. Therefore, from the direct support to agriculture in the form of increasing the level of subsidies, the whole society will benefit indirectly. In developed countries, the state intervenes in four ways: a) determines the appropriate pricing policy of agricultural products, b) determines the measures of accelerated inputs for different types of costs, c) determines the appropriate financing models for agricultural production and inventory, and d) subsidizes the export of agricultural products (Pejanovic *et al.*, 2003)

In Serbia, 2.43% of the total state budget was allocated for agriculture in 2012. It's a very small amount considering the benefits that can be obtained by increasing agricultural production with higher subsidies. The disbalance between the importance of agriculture to the economy as a whole and of its participation in the state budget can be illustrated by the fact that during 2001 the agriculture production rose by 20%, leading to an increase in GDP of 5%.

Table 1: Total subsidies from the budget in period 2006-2011 (in thousands of dinars)

Year	Budget Expenditures	Agriculture Subsidies	% of total Expenditures
2006	529,707,500	11,180,700	2.11
2007	617,625,300	12,754,300	2.06
2008	702,068,300	25,309,900	3.60
2009	746,454,700	16,694,300	2.24
2010	820,240,800	22,863,900	2.79
2011	877,295,100	18,020,100	2.05

Source: Serbian Chamber of Commerce and author's elaboration

Agriculture has a constant need of financial support. The funds must be invested in agricultural production at a time and on a large scale, according to the nature of production, long holding time commitments, and low profit of the primary agricultural production, which prevents the creation of its own accumulation and its own sources of funding. An agricultural policy known for a longer period than one year is imperative to Serbia, in order to create an environment characterized by stability, predictability and attractiveness for both domestic market participants and foreign investors.

Development can no longer rely on small producers, in a fragmented agricultural property, but above all Serbia needs large agrarian systems that are able to implement on their property modern technology and thus be in a position to properly counter the international competition and stabilize its position in the market. In developed agricultures the average property is more than 30 hectares, and in Serbia 10 times less. Serbia can easily slip into a situation where it starts to import more food in the coming years when, because of the Stabilisation and Association Agreement (SAA) and the accession to the World Trade Organization (WTO), the level of protection will be lowered. Exports will be increased, but not as fast as imports. Simply, it happened to all the other countries, so it will happen to Serbia as well. The European Commission itself has criticized the fact that the measures in agriculture have been ad hoc so far and often changed two or even three times a year.

It's hard to find a good reason why Serbia as an agricultural country, where the farmers have always been proud of their full barns and dryers, millions and millions of euros are spent on imports of fruits, vegetables, meat. It is very disturbing knowing that Serbia spent about 5 million dollars on imports of apples in 2009, although this fruit is produced in sufficient quantity. Apples had a good harvest, but much of the fruit remained on the branches, because the producers due to low prices didn't want to collect and to sell them. In addition, Serbian farmers still don't have a quality storage technology, so the traders choose to buy these fruits abroad, because it is more profitable. Apples are not the only problem, but beans as well, which will be unfortunately imported for a long time mostly from Kazakhstan, since one ton per hectare is low and insufficient, then tomatoes, peppers from Macedonia and Greece, onions from the Netherlands and Belgium, garlic from China. All this drastically affect the financial picture of the Serbia's balance of payments position. However, Serbian companies in the agri-food sector have great opportunities to increase exports of local products to Germany, Italy, France, since those countries are the largest importers of agricultural products from developing countries, including Serbia. Agriculture in Serbia contribute to national wealth with significant participation in the creation of GDP. The share of agriculture in GDP of Serbia in the period from 2001-2010 was in the range 24.6 - 9.7%. Such a high percentage is even more important considering that the value of primary agricultural products is significantly lower than the value in the food-processing industry, and points to the crucial importance of this industry to the overall national economy. The average

annual growth rate of net agricultural production in Serbia in the last 10 years was 1.3%, and the gross value 1.9%. The last 10 years, agriculture is going through a transition phase, and the entire Serbian economy as well, which involved privatization process that has been proceeded without adequate and clearly defined measures of agricultural development policy.

Currently only competitive advantages of Serbia are the natural resources, low labor costs, low land prices (Tomic, 2011). However, available resources (favorable natural and climatic conditions) are not sufficient for the survival of agriculture and sustainable rural development. It is necessary to support the development of agricultural and rural policy with adequate and intensive budget allocation. Some of the measures to increase the competitiveness of Serbian agriculture could be divided into the following categories:

- The participation of big commodity producers should be increased in the structure of agriculture, as currently dominated by small commodity producers. The big commodity producers would increase the productivity.

- Reorganization of the market of agricultural products in order to reduce the monopoly on the demand side, as well as to price stability and competitiveness in order to place products outside of Serbia.

- Increasing agricultural budget with a bigger participation of subsidies.

Table 2: SWOT analysis of agriculture in Serbia

<b>Strength</b>	<b>Opportunities</b>
<ul style="list-style-type: none"> <li>- Natural resources</li> <li>- Unpolluted resources</li> <li>- Educational institutions</li> <li>- Manufacturing facilities</li> </ul>	<ul style="list-style-type: none"> <li>- Merging of land properties</li> <li>- Improvement of production</li> <li>- Multifunctional production</li> <li>- More efficient utilization of land</li> <li>- The larger volume of organic production and products with protected geographic origin</li> <li>- Development of cooperatives</li> <li>- Education of the local population</li> </ul>
<b>Weaknesses</b>	<b>Threats</b>
<ul style="list-style-type: none"> <li>- Fragmentation of property</li> <li>- Elderly households</li> <li>- Problems in irrigation</li> </ul>	<ul style="list-style-type: none"> <li>- Restrictions on exports</li> <li>- Lack of state support</li> <li>- Insufficient impact of institutions on development</li> </ul>

Source: Author's elaboration

In the past few years Serbia had stable growth, the average GDP growth rate for 2006/2007/2008 was around 6% (Table 3). In 2009, however, mainly due to global economic crisis and its effect on Serbian exports and investments in the country, a negative growth and contraction of GDP was recorded (-3%). The largest influence on the GDP contraction had the fall in agricultural production of 20% due to the severe summer drought. In table 3 we can see that the share of agriculture in the total import and in the export as well, still has fluctuations and indicates the instability of the agricultural sector, as a result of absence of a stable agricultural policy. The data in the table also show the instability in spending on subsidies in agriculture and although there is some small increase (2006/2007/2008), this increase does not

correspond to the relative trend and the need for development of small farms and the preservation of rural areas. Therefore, in reality the downward trend and from 2009 the sharp decline of the agricultural budget in the national budget has been evident. How the share of the agricultural budget in total government budget was reducing, at the same time the share of agriculture in GDP was decreasing, and thus influenced the issue of financing the trade deficit. Decrease in the share of agriculture in GDP has been affected also by the development of other sectors of the economy, which however do not have such a positive performance in reducing the negative trade balance as agriculture has. Serbia did not set a hard line on the need of continued spending on subsidies, as well as setting the pace and terms of assistance to agriculture and farmers. The very fact that the agriculture in Serbia accounts for about 9% of gross domestic product, and the agriculture budget with approximately 2.48% in the overall budget (data for 2010) – presents itself the state's attitude towards agriculture. Given the still low profitability of agriculture in the Republic of Serbia, the main source of funding and support for agriculture is still in the hands of the state. No agricultural producers can survive without protection and subsidies, regulated by the state, and yet the same have recorded a tendency to a steady decline.

Table 3: Trade balance; Agriculture trade balance; GDP - Serbia (in mil.USD)

	2006	2007	2008	2009	2010	2011
<b>Total export</b>	6,431	8,823	10,974	8,345	9,794	11,780
<b>Total import</b>	13,174	19,165	24,332	15,808	16,471	19,862
<b>Trade balance</b>	-6,743	-10,342	-13,358	-7,463	-6,677	-8,082
<b>Agriculture – export</b>	1,265	1,686	1,957	1,945	2,241	2,700
<b>Agriculture – import</b>	905	1,116	1,468	1,308	1,036	1,400
<b>Agriculture – trade balance</b>	<b>360</b>	<b>570</b>	<b>489</b>	<b>637</b>	<b>1,205</b>	<b>1,300</b>
<b>Agriculture - % of total export</b>	19.7	19.1	17.8	23.3	22.9	22.9
<b>Agriculture - % of total import</b>	6.9	6.1	6.0	8.3	6.2	7.0
<b>Agricultural budget - % of total budget</b>	4.70	3.60	4.00	2.20	2.48	2.45
<b>GDP growth rate</b>	5.2	6.9	5.5	- 3.0	2.0	1.6
<b>Agriculture - % of GDP</b>	11	10	11	10	9	9
<b>Trade balance - % of GDP</b>	-21	-24	-27	-18	-17	-16

Sources: Statistical Office of the Republic of Serbia, The World Bank, Author's elaboration

### Conclusion

International trade today depends on many factors and is restrained by the effects of various types of barriers, which are constantly adopted and implemented by highly developed world economies. As a result there are some difficulties in the flow of goods between regions and groups of countries, affecting international flows of agricultural products. In this way, the traffic of goods directed to less economically developed countries and to those who are not members of a certain economic group is transparently discriminated. In addition, beside this type of agricultural protectionism, in the international market operates a number of

multinational companies, the agricultural clusters, with which it is difficult to establish a fair competitive battle in the market.

Favorable global financial conditions prior to the global financial crisis allowed the smooth financing of the deficit of Serbia's current account using foreign sources of funding. At the same time, the external debt of the private sector has been also increased, which is why the economy has become vulnerable to sudden reduction of capital inflows, which greatly influenced the increase in the budget deficit. One of the most significant sources of inflows in the Serbian economy certainly can be and proceeds from the export of agricultural and food products. As in recent years, the funds in the agricultural budget have recorded a relative and absolute decrease. It is suggested that in 2013 the agricultural budget should be 5% of the total national budget, and in the coming years this share should reach 10 %. The priority of the Serbian agriculture is to increase the general level of competitiveness, find new markets, adapt to the rules and standards of the EU and the WTO, acquire new skills and technologies that will change the structure of agriculture and be ready for the competition in the domestic and international markets. One of the prerequisites for establishing the long-term macroeconomic stability in Serbia, which is characterized by a sustainable level of public debt, price stability, reduced trade deficit and unemployment, is certainly the formulation of the development concept for agriculture, the development of institutions, legislation, subsidies and investments in agricultural resources.

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**PERSPECTIVES OF RURAL TOURIST DESTINATION MOUNTAIN  
RAJAC I VRANICA**

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**Abstract**

The paper describes a cross-border project, which is funded by the European Union under the Instrument for Pre-Accession Assistance (IPA). The aim of the project is to strengthen the rural economy by supporting the professional development of rural tourism in Serbia and Bosnia.

Landscapes Rajac and Vranica are similar, both in their tourism offers and the growth potential. Both regions have been known tourist attractions, picturesque rural tradition and well-preserved natural resources. The project aimed to create a strong cross-border cooperation between the two regions, and to support the development of rural tourism destinations Rajac and Vranica.

The development of tourism in the villages of central Bosnia and Herzegovina, and Serbia has the potential to increase the diversification of the rural economy, particularly the establishment of national cooperation. The main objective of the project is to ensure that the impact will go beyond strictly defined border areas and help the two countries to put into operation and capitalize on all their rural tourism resources.

**Keywords:** cross-border cooperation, rural tourism, Instrument for Pre-Accession, Rajac, Vranica

**Introduction**

The project “Support to the development of rural tourism destinations Rajac and Vranica“ was developed to respond to the efforts of the EU for cross-border cooperation between Serbia and Bosnia and Herzegovina, and to contribute to solving problems in rural areas, such as low levels of regional exchanges, the reduction of poverty and the negative trends of rural exodus, inadequate access to markets, protection of natural local identity and cultural heritage.

Support for rural tourism is an effective way to alleviating these problems. Target areas were selected Ljig-Serbia (already established as a destination for rural tourism), the municipality Fojnica of Bosnia and Herzegovina (already established as a spa and ski tourist destination), while the municipality Kresevo of Bosnia making the first steps towards the development as a tourist destination. The goals should be achieved through: professional education of tourism entities, renovation and rehabilitation of historic buildings and improving the tourism infrastructure, creating promotional materials, improving on-line access to the market and the establishment of cross-border networks in rural tourism.

Applicants in the project “Caritas International Belgium“ and Association “Rural tourism Serbia“. Other partners were: Caritas Bishops' Conference of Bosnia and Herzegovina, Kresevo municipality-Bosnia, Caritas Serbia and Tourist organization Ljig-Serbia. The CBC<sup>42</sup> programs financed under the Instrument for Pre-Accession Assistance

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<sup>42</sup> CBC - stands for cross-border cooperation

(IPA), which provides support for the candidate and potential candidate countries on their way to European integration. Cross-border Programme Serbia - Bosnia and Herzegovina, seeks to promote sustainable economic and social development and to improve the connection between the program area.

### **ethods for project implementation**

The aim is primarily to identify underutilized potential for the development of rural tourism, mountain tourism destinations Rajac and Vranica, and point out the flaws that prevent the growth of this type of tourism.

The development work will be used the following research methods: historical method, the method of content analysis; mapping method, the method of comparison; interview method and the method of synthesis reasoning.

The expected results of this study are to promote rural tourism destinations and Rajac Vranica at the national, regional and European tourist market. Taking into account the level of research for this study, the expected contribution will be the identification of tourism products and destinations Rajac Vranica and identification of tourist attractions and potential for rural tourism development .

The rural tourist destinations Rajac and Vranica with natural resources, cultural and historical heritage of traditional architecture, cuisine, folk traditions, local crafts, native livestock breeds and plant varieties have all the potential for the development of rural tourism. The offer of rural tourism in the two areas are villages, ethnic villages, ethnic houses and events that offer tourists an authentic experience of rural environment .

### **Providing advice and financial support to communities using the CBDA<sup>43</sup> (CommunityBased Development Approach) methodology**

Advisory support (capacity building) will be provided through training, workshops and conferences and they can cover three main topics:

- Application CBDA approach (mobilization of local resources, facilitating meetings with community representatives),
- Management (proposals, strategic planning, financial management, etc.)
- Specific expertise (tourism, agriculture, poverty reduction, etc.).

The mobilization of local resources is a process in which members of the community together and contribute to the distribution of available funds, materials and services (facilities and equipment), through volunteer work and media coverage in order to meet the identified needs. Financial support should be most focused on direct action in the community, while the other portion of the funds should be allocated through small grants (organizations and individuals).

The criteria for the allocation of shared resources can be:

- The level of participation in the project planning process,
- The number and diversity of human resources that go planning meetings,

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<sup>43</sup> CBDA (Community Based Development Approach) - The “development approach based on the community“.



- Local ownership of project ideas,
- Project from within the local community and is recognized as a local need or interest of the majority of community members,
- The number of people in the community interested in the project,
- Local governments, businesses and other local organizations interested in contributing to a project (material resources, volunteers, media coverage),
- The willingness of local authorities to provide financial support.

The agriculture sector is still the biggest opportunities for the rural population to overcome the difficult situation. Studies “Assessment of tourism potential of the municipality of Kresevo”, “Preliminary study on rural B&B<sup>44</sup>’s in Bosnia and Herzegovina” and “A Study of Rural Tourism in Bosnia & Herzegovina”, show that the growth generated by agriculture is up to four times more effective in reducing poverty than growth in other sectors.

Linking small farmers with good functioning of local markets - plays a key role in long-term poverty reduction. Model linking small producers in the market, and identify markets that use certain types of manufacturers, are critical steps for the development of cross-border projects. Formal requirements of the market, including the quality, consistency, food safety, require direct communication and coordination in the supply chain of agricultural and food products. While these market requirements are not formal barriers to entry of new producers, especially those with fewer resources, they will still represent potential opportunities for diversification, income and the professionalisation.

#### **Steps involved in the adoption of CBDA:**

- Involvement of experts to develop a detailed methodology of CBDA with additional tools (access, principles, activities, methodology, mechanics, school programs, etc.),
- Design and build a model of the Caritas CBDA methodology,
- Educate staff about the practical implementation of Caritas CBDA methodology (training of trainers),
- Through a pilot project to implement a methodology in three selected communities, with monitoring by experts,
- To adopt the methodology of CBDA as a strategic tool Caritas, for project,
- CBDA methodologies - a factor in the success of the project.

CBDA methodology is the fastest growing mechanisms for channeling development assistance. CBDA term is a term that refers to projects that actively include beneficiaries in their design and management. This methodology has been implemented in Bosnia and Herzegovina in various ways since 2000, at the beginning of international NGOs<sup>45</sup> such as Caritas and others., and later adopted by local organizations. Today, this methodology is integrated into the mission of many community organizations and is used in most of the projects supporting social and economic development of communities in Bosnia and Herzegovina.

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<sup>44</sup> **B&B** - an acronym “bed and breakfast“, is a small lodging establishment that offers overnight accommodation and breakfast, but usually does not offer other meals.

<sup>45</sup> **NGOs** - non-governmental organizations, are legally constituted corporations created by [natural](#) or legal people that operate independently from any form of [government](#).

**Expected outcomes of the project would be as follows:**

- ✓ Developed sector of rural tourism in both areas by creating new and innovative tourist products;
- ✓ Strengthening the competitiveness of rural tourism stakeholders in both territories, through the improvement of professional skills;
- ✓ Promotion of the rural tourism in both areas, using a new marketing and ICT<sup>46</sup> tools;
- ✓ established sustainable regional cross-border networks of rural tourism for better representation at the European level.

**Results and discussion**

The planned activities of each partner led to improve the capacity of target communities to attract and receive tourists. Activities were carried out in the range from setting up tourist signs marking the trail (hiking and biking), the creation of themed travel to the reconstruction of buildings and protection of cultural heritage, and so on.

Marking and signposting of 10 footpaths in the length of 90 km in the territory of Serbia Rajac mountain was completed in collaboration with the Mountain association “Pobeda“ from Belgrade. Marking and signposting on footpaths 7 with a total length of 100 km in Bosnia, in the mountains Vranica was completed in collaboration with two mountaineering association “Vranica“ from Fojnica and “Bitovnja“ from Kresevo. Map of all these roads were designed and printed for free distribution among tourists. Information signaling panels were installed and are available to tourists and residents in the municipality Ljig. A total of 45 km of cycle paths is marked in Serbia. Marking the loop that makes the route length of 30 km of local roads in Kresevo also completed. The project has developed two new themed tours in Kolubara District, with labeling panel and two folders named “Rakija“ and “Kajmak“. Two thematic tours have been designed in the municipality Kresevo in an area called “Bosnian soul and spirit“ and “mouth full health“. Brochures two thematic sections are printed, and promotion conducted by the Tourism organization of Travnik.

During the project was the rehabilitation of 2 buildings of the old architecture of municipalities Ljig and Kresevo. Project funds restored chalet on the slopes Rajac “Dobra Voda“ which run by the MA “Pobeda“. In the war was reconstructed blacksmith’s workshop, which will be used for special occasions, such as for a demonstration and preservation of traditional crafts.

Platform and equipment for paragliding and meteorological instruments were purchased and installed on the mountain Rajac (1 platform/shelter, 10 sets of paragliding equipment, 1 weather station). Information about flight conditions (wind speed) can be obtained by phone, from people who work in the mountain hut. The implementation of the Project activities established recreational area on the mountain Rajac and purchased 13 bicycles for eco association. In the center of Rajac is a wooden hut for kids and installed with 7 wooden playground equipment. Also, funds project procured equipment for hiking MA “Vranica“ and MA “Bitovnja“ in the municipality Kresevo.

Trainings and workshops are a good way to improve the knowledge and skills of the actors. Each workshop is designed as 2 days of learning and education. Workshop activities are created trainers who are leading experts in the relevant field. For workshops focused on specific skills such as “Traditional gastronomy workshops“ participants were those who are using newly acquired knowledge in their daily activities. The workshops were attended by representatives of tourism organizations that have supported this form of education. After training were organized and planned study tours, new trainings and workshops. Published two

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<sup>46</sup> ICT- stands for Information and Communication Technologies

bulletins on rural tourism in the blog<sup>47</sup> [vranicarajac.wordpress.com](http://vranicarajac.wordpress.com). The first newsletter was published in december 2011, while the second was published in june 2012.

Promotional activities have been carried out via the website [www.selo.rs](http://www.selo.rs). The site is improved and the number of services that are advertised on the site has increased. The site is very successful, and association “Rural tourism Serbia“ establishes quality partnerships with tourism organizations in each municipality in Serbia and in the region. Serbian national portal [selo.rs](http://selo.rs) is enhanced with an updated database of available services. It is a second generation site and is fully operational. It also increased the number of new services that are advertised on the site. Promotion of rural tourism in Bosnia and Herzegovina at the beginning. Site created by the project participants [www.alterural.ba](http://www.alterural.ba) first of its kind in Bosnia, so that all the work has to start from the ground up. Although there are not many services that are advertised on the site, it's a great promotional site with excellent prospects for growth in the future.

Logos Rajac and Vranica are designed and approved by the parties involved. Logos destinations are now being used on the cards, flyers and other promotional materials. Network cross-border cooperation members provide stability and thus create a greater potential for creating and strengthening partnerships, they can be useful for those who choose to join.

The project has provided excellent networking opportunities through joint workshops, although to the relatively small number of people. These Studies are made iduring the project, they are very good documents, but very little to promote cross-border networking. In contrast to the study, “blog“ has contributed to a much better and more networking. Blogs and newsletters support cross-border cooperation and networking, as in the creation of texts included both the applicant and other partners from both countries. The blog is also available on the Internet still exists: [vranicarajac.wordpress.com](http://vranicarajac.wordpress.com).

Table 1. Promotional materials presented and distributed during the project

Materials	No.
Project inauguration leaflet	2.000
Folders	300
Pens	300
Banner	1
Ljig tourism brochure	1.000
Regional rural tourism map	1.000

*Source:* Association “Rural tourism Serbia“;

Promotional materials were presented and distributed during the life of the project (Table 1.) represent a very useful activity that despite financial expenditure gives the results for a longer period, as follows:

- a) Brochure “Tourism in Ljig“ was distributed at tourism fairs in Belgrade and Novi Sad, the stand of the Tourist organization of Ljig over Mountaineering society “Pobeda“ in Belgrade;
- b) Map “Rural tourism“ was presented and distributed at the Tourism fair in Belgrade and tourism organizations across the Gornji Milanovac, Kosjeric, Ljig, Mionica and Valjevo;
- c) Inaugural leaflet distributed during the opening ceremony of the project on Rajcu is given to

<sup>47</sup> **Blog or weblog** (blog for short, of Weblogs, forums) - is a series of chronologically organized text input, which is presented on web pages (mainly the entries are sorted from newest to older) through automated software that enables very easy creation and blogging.

partners from Bosnia, for further distribution (Caritas BK BiH), as well as reporters during a promotional trip.

### **Conclusion**

The project “Support to the development of rural tourism destinations Rajac and Vranica“ has been developed in order to strengthen cross-border cooperation between Serbia and Bosnia and Herzegovina. Furthermore it aims to contribute in solving the growing problems in rural areas. In addition, the project supports the development of rural tourism destination on the mountain Rajac and Vranica.

These objectives are achieved through: professional education tourism entities, reconstruction of historic buildings, improvement of tourist facilities, creation of promotional materials, improving online access to the market through the establishment of partnerships between stakeholders.

The main impacts of the project are: setting up rural tourism as a potentially valuable component of community development, to raise tourism (in terms of improving infrastructure, equipment and services), improving market access (through very good sites) and of local and cross-border cooperation.

### **Acknowledgement**

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## GROUP DYNAMICS FOR POVERTY ALLEVIATION IN PAKISTAN

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### Abstract

Community Organization has been considered as one of the major preconditions for successful poverty alleviation in developing countries. It led the Pakistan Poverty Alleviation Fund (PPAF) and 19 of its partner organizations to implement a program to organize about one million households in 50,000 Community Organizations (COs) in 25 poorest districts of the country. The study analyzed these COs for the process of their formation, gender dimension and the readiness of office bearers or developed leadership cadre to mediate the local development that matters to them. A sample of about 5,213 COs with a confidence level of 99 percent and an error margin of  $\pm 2$  percent was surveyed using a questionnaire that asked qualitative and quantitative information on the said aspects of community organizations. The findings revealed that the democratic processes in these organizations have yet to be evolved as the office bearers on top ranked positions in almost all COs were selected instead of election process. In away, the gender dimension has been well taken care of during the group formation process as 55 percent of the COs correspond to female, 40 percent to male while the remaining 5 were mixed of both gender. Most of these organizations have reported community savings at the time of survey. However, on average a group of 16 households could not save more than 50 USD – an amount certainly inadequate to start lending operations. Besides, some increase for both males and females members having Computerized National Identity Cards, vote and marriage registration could be attributed to the emergence of COs. About three-fourths of the community organization reported having development plans while one third having linkages with public and private organizations and Village Organization. Nevertheless, only 6 percent of COs joined the Local Support Organizations – networks at sub-district level. Last but not the least, trainings seems to have contributed to smooth functioning and record keeping of COs although the decision regarding the training were mostly done by the Social Organizers of Partner Organization instead of the communities on their own. The study has useful policy implication for the empowerment of the poor by organizing them at household, village and Union Council level to build their voice and scale for an effective interface with government and market.

**Keywords:** Community Organization, Poverty alleviation, Local Support Organizations, Village Organization, Pakistan Poverty Alleviation Fund

### Introduction

Community organizing is a process where people who live in proximity to each other come together into an organization that acts in their shared self-interest. A core goal of community organizing is to generate durable [power](#) for an organization representing the [community](#), allowing it to influence key decision-makers on a range of issues over time. In the ideal, for example, this can get community organizing groups a place at the table before important decisions are made. Community organizers work with and develop new local leaders, facilitating coalitions and assisting in the development of campaigns.

Organized community groups attempt to influence government, corporations and institutions, seek to increase direct representation within decision-making bodies, and foster [social reform](#) more generally. Community organizers generally seek to build groups that are democratic in governance, open and accessible to community members, and concerned with the general health of the community rather than a specific interest group. Organizing seeks to broadly [empower](#) community members, with the end goal of distributing power more equally throughout the community.

The three basic types of community organizing are [grassroots](#) or "door-knocking" organizing, [faith-based](#) community organizing (FBCO), and [coalition](#) building. [Political campaigns](#) often claim that their door-to-door operations are in fact an effort to organize the community, though often these operations are focused exclusively on voter identification and turnout.

In Pakistan, various national and international organizations have been involved in the process of formation, capacity building and providing financial and technical support to community based organizations (CBOs) and groups of citizens since 1990. With the support of Pakistan Poverty Alleviation Fund (PPAF), more than 50,000 community organizations were developed under a project ended during June 2010. In order to know the existence as well as capacity to carry out activities envisaged for community organizations, a sample of about 5,000 community organizations was selected and surveyed. Estimates of the survey reveal the level of competency of community organizations and assess the training needs for their development and progress.

## **Material and methods**

### **2.1 Research Design**

The primary purpose of this study was to verify the existence, quantify some selected indicators describing strengths and weaknesses of community organization and evaluate the progress before closing the Project i.e. June 2010. Therefore, name of the design used for this study is descriptive research with special reference to descriptive survey. Descriptive survey is appropriate for obtaining people's perceptions on social issues and social facts concerning the current status of phenomena and/or for describing the nature of existing conditions in a situation (Cohen and Manion, 1980; Trochim, 2000).

### **2.2 Population / Area of the Study**

This study reflects all the community organizations in 25 poorest districts spread over 4 provinces of Pakistan, namely Punjab (56%), Sindh (21%), Khyber Pakhtunkhwa (14%) and Balochistan (9%). Total number of community organizations is reportedly more than 50,000 covering one million households. These organizations were developed with the support of Pakistan Poverty Alleviation Fund under the project ended during June 2010.

### **2.3 Sample Size and Sampling Method**

In all, 5,213 community organizations, which constituted about 10% of the total, were surveyed; which reflected population parameter at less than 5% error rate (Wunsch, 1986). Distribution of the sample by province reflected as 2,894 (56%) from Punjab, 1079 (21%) from Sindh, 748 (14%) from Khyber Pakhtunkhwa (KPK) and 492 (9%) from Balochistan. A sample of community organizations were selected using 2 stage cluster sampling method. Provinces and districts were considered clusters at the first and second stage, respectively. From each cluster, a random sample of community organizations was selected using systematic sampling method whereby every  $K^{\text{th}}$  item is selected from sampling frame of community organizations (Anderson *et al.*, 1993).

### **2.4 Development of Questionnaires**

Research team of working within the subject area of social sciences developed indicators particularly focusing on general information to verify the existence of social organizations; selection procedure of key persons; details of saving and utilization; record keeping;

awareness and training programs; proactive initiatives; access to public and private sector offices; and impact of training programs.

Closed ended questions were developed on the above mentioned indicators; thus, a questionnaire was designed for survey of community organizations. The fundamental principle of the questionnaire was taken in consideration i.e. keep it simple and short (KISS). Necessary changes were made during pilot survey.

Impact of training programs on three subject areas viz. conducting meetings, planning and record keeping was measured using 5-point Likert Type Scale (1 being poor, 2 being average, 3 being medium, 4 being good, and 5 being excellent) was used.

### **2.5 Training of Enumerators and Data Collection**

Data were collected by 25 enumerators particularly hired for the present investigation. Prior to data collection, a training session was conducted on the questionnaire. At the first hand, each and every question was discussed in detail. Enumerators were asked to fill in the questionnaire hypothetically. After making possible corrections, the enumerators were asked to collect data from community organizations as a pilot survey. Supervisors checked the quality of data and passed instructions to the data collection team from time to time.

### **2.6 Statistical Analysis**

Data collected from the field was checked thoroughly and fed in Microsoft Excel package. Using filters, data were cleaned for outliers and influential observations. Data were transferred to Statistical Package for Social Sciences (SPSS) and the same were analyzed. Descriptive statistical methods namely frequencies, percentages, minimum, maximum, and mean were computed.

## **Results and discussion**

### **3.1 Selection Procedure**

Selection of the office bearers through election was rarely reported as only 1% organizations declared. It was reported that active members of community organization are selected as office bearer particularly on the top ranked positions namely president, vice president, and secretary.

### **3.2 Recording of Selection Procedure**

Recording of selection procedure was informed by overwhelming majority of the community organizations (97%) while the remaining 3% had no any record in this regard. Disproportionately, more number of community organizations having no any written record/minutes of the selection was found in Balochistan. This may invite attention that capacity building programs for the community organizations may be launched especially in Balochistan. Except Balochistan and KPK, recording of selection procedure was observed in almost all the organizations in Punjab (99.6%) and Balochistan (99.6%).

### **3.3 Type of Community Organizations**

On an overall basis, female organizations were found in majority (55%) followed by male (40%) and mixed (5%). Traditional variations are crystal clear when data were segregated on provincial basis. There were 77% female community organizations in the Punjab against 6% in Sindh, 2% in Balochistan and only 0.5% in KPK. During the last many years, development of female organizations in KPK and Balochistan remained stagnant especially due to poor law and order situation and illiteracy in rural areas.

### **3.4 Average Number of Members by Gender**

On an overall basis, there were 16 male members in an organization while the female number was almost the same (15%). The highest average (about 20 members) number of male members was found in KPK followed by Sindh (18 members), Balochistan (16 members) and Punjab (11 members). Like male membership, exactly the same trend across the provinces



was also recorded with the highest average number of female members in KPK (18 members) followed by Sindh (17 members), Balochistan (16 members) and Punjab (15 members).

### **3.5 Proportion of Poor Households in Community Organizations**

Average number of households organized in each community was about 16; thus total population was enumerated about 80,000 in more than 5,000 community organizations. Out of these, about 51,500 were categorized as poor regardless of operational definition of poverty. However, poverty in terms is considered as lack of money to purchase especially food items and lack of access to education and health facilities. The proportion of poor households was computed as 64%. The highest poverty level was recorded in KPK (79%) followed by Balochistan (69%), Punjab (62%) and Sindh (54%).

### **3.6 Frequency of Meetings**

On an overall basis, meetings were organized on monthly basis as reported by 82% of the community organizations while the remaining 17% reported that meetings are organized fortnightly. Frequent meetings (fortnightly) were relatively more common in Sindh (29%) and Punjab (20%).

### **3.7 Whether Poverty Cards Filled**

On an overall basis, about 13% of the community organizations reported that the poverty cards were filled. Remarkably more proportion (33%) of community organizations of Balochistan province reported that poverty cards were filled. About one-fourths (25%) of the community organizations of Sindh province reportedly filled poverty cards.

### **3.8 Average Savings Per Community Organization**

On an overall basis, 92% of the organizations reported to have savings. This ratio increased to 96% for Punjab, followed by KPK (92%), Sindh (82%), and Balochistan (81%). The average saving per community organization was 3,263 Pakistan Rupee (Rs.), on an overall basis. The highest average (Rs. 4,776) was computed for Sindh province. Although Punjab had more proportion of COs reported savings, the average amount (Rs. 2,741) was minimal in comparison of other provinces.

### **3.9 Bank Account of Community Organization**

On an overall basis, only 42% of the community organizations had Bank accounts. This proportion varied from 10% (Sindh) to 63% (KPK). There were about 54% community organizations in Punjab and 17% in Balochistan having bank accounts. From these figures, it may be concluded that these organizations were very much formal in financial dealing because of bank accounts.

### **3.10 Average Amount of Internal Lending**

About 16% of the organizations reported internal lending while the average amount was Rs. 2,486. Little less than one-fourths (24%) of the community organizations of Punjab reported internal lending with average amount of Rs. 2,242. Very small proportion (1%) of the community organizations of Balochistan offered money to their members with a minimum amount of Rs. 1,466.

### **3.11 Beneficiaries by Gender of Internal Lending**

About 2% of the community organizations were found in internal lending to male members while 14% of to female members. Very small number (1.9 males and 1.3 females) of members could be captured as beneficiaries of the internal lending. Interestingly, Balochistan had small proportion of organization with internal lending while the average number (3.14 males and 14.0 females) of beneficiaries was relatively larger than other provinces.

### **3.12 Beneficiaries by Gender of Internal Lending**

On an overall basis, 6% of the community organizations used their savings for different purposes. Segregation of data by province revealed that 13% of the organization in Balochistan used savings of community organizations while the minimum proportion (1.1)

was recorded for KPK. Proportion of community organizations of Sindh and Punjab was 11% and 5%, respectively.

### **3.13 Record Keeping of Community Organization**

Record keeping is one of the most important indicators to know their organization development. Initial training programs for the capacity building of the community organizations are related to record keeping. Record of members was reported by 84% of the community organizations, on an overall basis. This ratio was the highest (93%) for KPK followed by Punjab (85%), Sindh (80%), and Balochistan (71%).

There were 94% of the community organizations with meeting record. Proportion of community organizations possessing meeting record was the highest (97%) for Punjab while 88% of the meetings were correctly recorded. These estimates reflect that quite good efforts were taken for the capacity building of community organizations surveyed. Relatively weaker position was observed for COs of Balochistan where 69% of the COs shared record of 82% meetings. Almost the same estimates were recorded for attendance record. Relatively less proportion (70%) of COs with saving record was found in COs of KPK.

### **3.14 Members having CNIC<sup>48</sup> and Registered Votes**

In comparison of male members, impact of joining community organization on making CNIC and registering votes is very much obvious. Before membership of community organization, 13.8 males had CNIC which increased to 14.9 after joining community organization. On overall basis, 8% growth rate was calculated for male members while 43% for female members (from 8 to 11 members).

Likewise CNIC, growth rates in registration of votes for males and females were 5% (from 14.1 to 14.8 members) and 13.3% (from 8.3 to 9.4 members), respectively. From these figures, it may be inferred that community organizations are playing better role to get the women have right of citizenship and access to vote.

### **3.15 Married Members and Nikkah Nama<sup>49</sup>**

Average number of married members by gender and possessing Nikkah Nama before and after joining community organizations was estimated. It was informed that there were 14.2 married members of community organizations. Out of these, 14.1 were married even before joining COs. In other words, only 1% male members got married after joining community organizations. Relatively more proportion (2.7%) of females was recorded who got married after joining community organizations (12.2 before CO and 12.6 after CO).

There were 8.4 male members who had Nikkah Nama before joining community organizations and the same increased to 8.5 after joining; hence, the growth rate was 1.3%. For females, the growth rate for Nikkah Nama was 2.6% (average number of 9.7 before CO and 10 after CO). Moreover, it was recorded that 60% male and 80% females had Nikkah Nama.

### **3.16 Self Help Based Initiatives Taken by Community Organization**

One of the prime goals of community organizations is to develop the capacity building so that self based initiatives may be taken. However, the collected estimates show that there is a lot of room to enhance capacity of these organizations for solving their problems themselves without looking at others. On an overall basis, about 29% of the community organizations under this survey reported that self help based initiatives are taken by community organizations. This proportion ranged from 26% (recorded for Punjab) to 35% (estimated for Sindh).

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<sup>48</sup> Computerized National Identity Card

<sup>49</sup> Registered Marriage Contract

### **3.17 Members Development Plan**

Proactive initiatives of community organizations can be gauged from perception regarding development plans. On an overall basis, about three-fourths (74%) of the community organization reported that they had development plan while 58% of the all organizations had evidence. Settlement/community development plan was reported by 48% organizations which reduced to only 39% organization with some evidence.

### **3.18 Linkages Developed by CO with Public and Private Organizations**

Public and private sector partnership for the development of the country is being encouraged at all levels. At the grass-root level, community organizations contact various offices for organizing various programs. On an overall basis, 30% of the community organizations reported that they had linkages with public and private organizations. This proportion ranged from 13% (Sindh) to 37% (Punjab). Estimates are evident of poor linkages of the community organizations of Sindh with public and private sector organizations/offices.

Relatively community organizations of the Punjab had better linkages with almost all the public and private organizations at different tiers. Disproportionately more percentage of community organizations of Balochistan and KPK had links with local and international NGOs.

### **3.19 Selection of Community Organization Members for VO/LSO**

The primary function of the Local Support Organizations (LSO) is to support community organizations social mobilization and serve as a technical advisor for large development projects that are beyond the capacity of village or community level organizations.

On an overall basis, about one-thirds of the organizations were member of village organizations (VOs) while only 6% were members of Local Support Organizations. About 46% of community organizations of KPK were members of Village Organizations followed by Balochistan (36%), Punjab (31%) and Sindh (31%). Interestingly one of the organizations of Sindh province was member of Local Support Organizations against 10% in Balochistan and 9% in Punjab.

### **3.20 Perceptions Regarding Clarity of Objectives**

On an overall basis, majority (54%) of the respondents were of the view that objectives of training were clear to them while 39% rate as partially clear and 7% categorically opted “not clear”. The highest proportion of respondents with the clear objectives were recorded in Punjab and KPK (56% for each).

### **3.21 Decision Maker for Attending Training Programs**

Social Organizers (SOs) are the frontline extension agents involved in social mobilization and development of community activities. On an overall basis, 76% of the respondents were of the opinion that SOs made decisions about attending training programs while 22% selected community organizations. Role of office bearers (1%) and members (1%) of COs was almost negligible (1%).

### **3.22 Perceptions Regarding Impact of Training Program**

Majority (72%) of the respondents were optimistic that training programs were very effective. Slight variations across the provinces were observed since the highest proportion was recorded for Balochistan (77%) followed by Sindh (73%), Punjab (72%), and KPK (69%).

### **3.23 Level of Impact of Training Programs**

Capacity building of the community organizations is one of the prime tasks of national and international NGOs for the sustainable rural development.

Before training programs; knowledge, information, and skills was estimated as poor for all the provinces. However, after training programs, the same was measured as between average and

medium for conducting meetings (2.4) and record keeping (2.4) while almost average for planning (1.9). No any remarkable variations were recorded across the provinces.

### **Conclusions**

Community organizers generally seek to build groups that are democratic in governance, open and accessible to community members, and concerned with the general health of the community rather than a specific interest group. Organizing seeks to broadly empower community members, with the end goal of distributing power more equally throughout the community. Organized community groups attempt to influence government, corporations and institutions, seek to increase direct representation within decision-making bodies, and foster social reform more generally. In response to the Social Mobilization Project (2008-2010), Pakistan Poverty Alleviation Fund (PPAF) has to form 50,000 Community level organizations covering 1 million households in 25 Poorest districts of Pakistan. Taking a sample of 10% of all community organizations, community organizations were evaluated based upon various quantitative and qualitative indicators. Remarkable findings of the revealed that selection of the office bearers through election was rarely reported as only 1% community organizations declared. Overwhelming majority of the community organizations (97%) had record of selection. Female organizations were found in majority (55%) followed by male (40%) and mixed (5%). There were 77% female community organizations in the Punjab against 6% in Sindh, 2% in Balochistan and only 0.5% in KPK. On an overall basis, meetings were organized on monthly basis. About 13% of the community organizations reported that they have poverty cards filled. About 92% of the organizations reported to have savings. The average saving per community organization was Rs. 3,263. About 42% of the community organizations had bank accounts, on an overall basis. About 16% of the organizations reported internal lending. About 6% of the community organizations used their saving for different purposes. There were 94% of the community organizations with meeting record. Growth rate in preparing CNIC was 8% for male while 43% for female. Growth rates in registration of votes for males and females were 5% and 13.3%, respectively. About three-fourths (74%) of the community organization reported that they had development plan while 58% of the all organizations had evidence. Settlement/community development plan was reported by 48% organizations which reduced to only 39% organizations with some evidence. About 30% of the community organizations reported that they had linkages with public and private organizations. About one-third of the organizations were member of village organizations while only 6% were members of Local Support Organizations. Majority (72%) of the respondents were optimistic that training programs were very effective.

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**DESIGN, IMPLEMENTATION AND COORDINATION OF AGRICULTURAL AND RURAL DEVELOPMENT POLICY IN BOSNIA**

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**Abstract**

Rural economy in Bosnia and Herzegovina (BiH) is increasingly diversified but is still strongly dependent on the primary sector. Governance influences agro-rural development policies impacts on rural livelihoods. Governance analysis focuses on institutions and structures dealing with decisions making and implementation. The paper aims at analysing design, implementation, governance and coordination of agro-rural development policies in the Republika Srpska (RS) and BiH. The paper is based on primary information collected by questionnaires and semi-structured interviews carried out in summer 2010 with representatives of public and civil institutions – including international organisations and donors - dealing with agricultural and rural development at state, entity and municipal levels as well as an extended secondary data analysis. The number of organizations engaged in rural governance is rapidly growing and their role in policy design and delivery is getting increasingly important. Vertical coordination between State level institutions with entities, cantons, regions, municipalities and non-state actors, especially civil society ones, is still particularly challenging in BiH. Coordination between the State Ministry of Foreign Trade; the Ministries of Agriculture of RS and Federation of BiH and the Department for Agriculture of Brcko district is crucial. Participation of civil society organizations in rural development policies design and evaluation should be encouraged. Governance model and arrangement should allow shifting rural development policy paradigm and practice from a sectoral to a multisectoral, territorial and integrated approach that fosters rural livelihoods and economies diversification. Effectiveness of vertical coordination also depends on horizontal coordination at RS and FBiH levels.

**Key words:** Rural development, Agriculture, Policy, Governance, Coordination, Bosnia.

**Introduction**

Bosnia and Herzegovina (BiH) consists of two governing entities, namely the Federation of Bosnia and Herzegovina (FBiH) and Republika Srpska (RS), and a self-governing administrative unit *i.e.* Brcko District (BD), under the State sovereignty. It goes without saying that this institutional and political setting influences not only the design and implementation of agricultural and rural development policies but also the governance of the whole country.

Rural economy in BiH is increasingly diversified, however, a significant share of households is still engaged in agriculture and the rural economy is still strongly dependent on the primary sector. Agriculture share in the gross domestic product (GDP) was 8.60% in 2010 (EC, 2011). Although some progress has been made, extensive production prevails which result, often, in outdated production techniques and low yields (MoFTER, 2009).

Agricultural and rural development can not be achieved without improving governance in Bosnian rural areas. Rural governance comprises mechanisms, institutions and processes of decisions making and implementation through which persons and groups articulate their interests, exercise their legal rights, meet their obligations and mediate their differences in rural areas (Cheema, 2005). Governance analysis focuses on the formal and informal actors involved in decision-making and implementing the decisions made and the formal and informal structures that have been set in place to arrive at and to implement decisions (Sheng *et al.*, 2007).

According to Uphoff (1986), institutions belong to the public, participatory, and private sectors. Rural groups and institutions can be also divided into user groups, service providing organizations and governance institutions. In order to contribute to good rural governance, local institutions and organisations should be participatory, consensus oriented, accountable, transparent, responsive, effective and efficient, equitable and inclusive and follow the rule of law (cf. Sheng *et al.*, 2007). Civil society sector in BiH is dominated by very small NGOs and women's and youth organisations (Sterland, 2004).

In BiH and RS, rural governance models are slowly experiencing a paradigm shift towards the concept of “the new rural paradigm” (OECD, 2006). Government support to the rural sector evolved from command-and-control policies under socialism to support for transition where donors have an increasingly important role. However, increased emphasis is needed on rural development support and improving public agricultural goods and services (Lampietti *et al.*, 2009). New models of local rural governance are emerging with shifting power and resources downwards from the state to the regional and local levels. The new models of local rural governance reflect a shift of rural development policies target from agriculture to a multisectoral approach, which also targets enhanced synergy between rural sectors and to create public-civil society-private partnerships (OECD, 2006).

The main objective of the paper is to analyse agro-rural development policies governance and coordination in BiH with a special focus on the RS.

### **Material and Methods**

The paper is based on an extended analysis of secondary information and on questionnaires and a semi-structured interviews with representatives of public and civil society institutions. Highly reliable secondary data from reports, research papers and statistical databases were used.

A questionnaire, focusing on the design and implementation of agro-rural development policies in BiH and on the evaluation of coordination between the involved actors, was sent by e-mail to around 120 representatives of different institutions and organizations. The questionnaire encompassed the main public institutions and civil society organizations dealing with rural development in municipalities, cantons, regions, and entities as well as at the state level. In particular questionnaires were sent to key actors such as the State Ministry of Foreign Trade and Economic Relations and the Ministries of Agriculture of the RS and the FBiH.

Additional information about local institutions dealing with agro-rural development were collected through phone interviews and meetings with representatives of some municipalities (Trnovo, Istocna Ilidza, Istocno Novo Sarajevo, Istocni Stari Grad, Pale, Sokolac and Rogatica) in Sarajevo-Romanija region (SRr) and of the town of East Sarajevo (TES).

## Results and Discussion

The design and implementation of agro-rural development policies involve different supra-national or international, national and sub-national actors (regional; intermediate or sub-regional; and local) (OECD, 2006). In BiH, intermediate levels, entities of RS and FBiH, have a crucial role in agro-rural policies design and delivery. International organisations and development agencies have implemented different rural development projects and programmes during the post-war period.

In BiH, all levels of governance, ranging from the state to municipal authorities, are involved in the agricultural sector management and rural areas development. At the state level, the most important institution that deals with agriculture and rural development is the Ministry of Foreign Trade and Economic Relations of BiH (MoFTER). The Sector for Agriculture, Food, Forestry and Rural Development is responsible for establishing a framework for the development of sectoral strategies, policies, programs and measures, and for their implementation aiming to harmonize agriculture development in the country.

The state policy in the agricultural sector, food and rural development in recent years is developed in accordance with the goals and needs for accession to the EU. However, establishment of main structures for receiving and managing the pre-accession funds is still a challenge. Systematic and structural harmonization of agricultural policies at the state level began with entry into force of the Law on Agriculture, Food and Rural Development of BiH, adopted in May 2008. The measures of the Law are basically classified into policy measures to support agricultural markets and measures for rural development. Policy measures to support agricultural market are divided in the measures to improve products quality, measures of direct support to agricultural farms and measures for foreign trade. Measures related to rural development encompass those measures aimed at increasing competitiveness, protecting rural environment, diversifying activities in rural areas and improving life quality in rural areas.

Moreover, the MoFTER, supported by the European Commission (EC), prepared the Strategic Plan for the Harmonization of BiH Agriculture, Food and Rural Development 2008-2011 and Operational Programme for the Harmonization of BiH Agriculture, Food and Rural Development 2008-2011. The key objective of the Strategic Plan is to provide a framework for the gradual harmonization of policies, programmes, institutions, laws, regulations, systems and services both within BiH and with the EU.

At the level of entities, institutions in charge of agricultural sector management are the Ministry of Agriculture, Forestry and Water Management in the RS and the Federal Ministry of Agriculture, Forestry and Water Management in the FBiH while Brcko District local administration has its own Department of Agriculture, Forestry and Water Management. In FBiH the system of responsibilities is further divided, so all 10 cantons have established departments for the issues of agriculture, veterinary medicine, forestry and water. The Strategic Plan for Rural Development 2009-2015 was adopted in the RS (November 2009).

Moreover the agricultural and rural sector is characterized by the presence of a number of international donors, such as the USAID, SIDA, Italian Cooperation, JICA, AECID (Spain), the European Commission, the World Bank, etc.

Financial support to individuals or companies involved in agriculture and rural development is provided also by micro-credit organizations and banks. The Federal Investment Bank and the Investment Development Bank of the RS have special kinds of credit lines aimed to support agriculture and rural development.

In the RS in general and SRr in particular, support for rural development by municipalities is partly stated in local planning documents, which include the Local Economic Development Strategies. Many local organizations operate in municipalities. Most of them have a

predominant charity character largely due to the consequences of the civil war. Sport, cultural, youth and students' organizations are present in a large number as well. Those involved in agro-rural development are mainly agricultural cooperatives, environmental associations, associations of entrepreneurs and cultural heritage preservation associations. Financial and technical support for NGOs and cooperatives is provided by local budgets. In all municipalities financial aid is guaranteed for those NGOs that are identified as organizations of public interest (*e.g.* organization of war veterans) while cooperatives and remaining NGOs have to submit specific projects to be eligible for funds from local and regional budgets.

Rural development strategies, plans and programmes are generally missing at local and regional level: Pale municipality has a strategy for development of agriculture, while in municipalities of Istocni Stari Grad and Istocno Novo Sarajevo preparation of this document was still in progress, and the other four surveyed municipalities (Trnovo, Istocna Ilidza, Sokolac and Rogatica) did not have any strategic document directly related to agriculture and rural development. Overall in the 2006-2010 period, local development strategies focused mainly on agriculture development rather than on rural development, however the trends, generally, show an increasing attention paid to non-agricultural activities. Nevertheless, it seems that there is a consistent lack of coordination between local institutions operating in rural areas.

About two-thirds (67%) of respondents to the questionnaire for evaluating the level of coordination between the actors dealing with agro-rural development policies in BiH were public institutions and 33% civil society organizations. Almost half of the respondents operate at local level (46%) while only less than a third (27%) operates at the state level. Some institutions operate at two or even three levels at the same time. However, some differences can be noticed between public and civil society institutions. In fact, public institutions are more present at the entity level (60%) than civil society organisations that are more present at the local level (60%). Most of respondents consider rural development as a cross-sectoral issue that includes the agricultural sector, while some of them provided their own definitions. All interviewed organizations are involved in design (67%), implementation (73%), and monitoring/evaluation (53%) of agro-rural development policies. Public institutions are mostly involved in design (90%), and less in implementation (60%), and monitoring/evaluation (60%). As expected, civil society organizations are fully involved in the implementation phase (100%) and less in policy design (20%) and monitoring/evaluation (40%). Almost all interviewees (93%) have had relationships with public institutions while most of them have had relationships with civil society and international organizations (86%). Only three fifths of the respondents evaluate the coordination among the different actors as effective. The main constraints and problems impeding good coordination are included in box 1.



Box 1. Major constraints hampering coordination of agro-rural development policies in BiH.

- Lack of knowledge and information
- Lack of communication among key actors
- Lack of qualified human resources in institutions and organizations
- Lack of understanding and of a common vision of rural issues and priorities
- Lack of clearly defined plans, initiatives and long term strategies
- Lack of funds
- Conflicts of interests
- Absence of a dialogue culture and participatory approaches
- Low use of information technologies
- Low attention paid to rural areas in the political agenda
- High level of administrative and bureaucratic requirements
- Delay in the establishment of the Federal Agency for Rural Development

Source: Authors' elaboration based on the questionnaire survey results.

Only 53% of respondents identified an organization as having the leadership in coordinating rural development issues. The institutions more widely identified as the most important in providing coordination of rural development issues are the Entity's Ministries for Agriculture while no public institution or civil society organization considered the Ministry of Foreign Trade and External Relations (MoFTER) as the leader institution regarding these issues. In fact, it is quite common in the decentralised or 'concerted' and multi-actors driven rural policy design and delivery systems (Mantino, 2009) that the different levels of government find it difficult to clarify their respective roles and responsibilities (OECD, 2006).

Respondents also mentioned some institutions with which they have had some conflicts. It is interesting to note that public institutions have mainly conflicts with governmental organisations and some international agencies while civil society organisations, also due to their nature, present a lower degree of involvement in those conflicts.

Overall most of the interviewees identified the main constraints in coordination among the different organizations dealing with rural development as political, technical and strategic. Some respondent also emphasized that in some cases competition is overcoming cooperation thus resulting in a major constraint.

The analysis of relationships and linkages between the institutions that are involved in the design and implementation of agro-rural development policies in BiH and RS showed a lack and/or weaknesses of coordination between them. Therefore, this problem should be addressed as soon as possible in order to increase the effectiveness of these policies and their impacts on rural people's livelihoods. A basic action to strengthen coordination would be to encourage dialogue between these institutions. While "formal dialogue" does exist between some public institutions especially those operating in RS and with some international NGOs and donors, it seems that a lot need to be done in order to involve civil society and private sector organizations especially during the design and formulation phase. That is critical

especially regarding the participation of rural people, farmers and their organizations. Developing strong partnership between national and sub-national governments through vertical governance arrangements and public-civil society partnering agreements can make Entity, regional and local governance institutions responsible by virtue of their participation in decision making regarding the design and implementation of rural development policies (OECD, 2006). Since local actors are called to perform new tasks, which requires changes in mind set and new skills, governmental institutions and international organisations should help local actors to acquire these new skills.

One of the obstacles that hamper coordination between civil society organizations and between them and public institutions is a lack of a common understanding of what is “rural” and what is “rural development”. The questionnaire suggested that despite the fact that all organisations consider rural development as cross-sectoral they mean different concepts when they talk about rural development therefore they use different approaches and they have different priorities which make difficult to have a common vision.

In order to strengthen coordination and synergy between institutions in promoting sustainable agriculture and rural development in RS it is also necessary to harmonize entity laws and regulations with the Law on Agriculture, Food and Rural Development of the BiH, that is, to a certain extent, in line with the rural development measures of the EU. That can make it easier also for international donors and NGOs to formulate their strategies for agro-rural development in all BiH.

Human capital has also a strategic relevance in order to achieve good coordination between involved institutions. In fact, institutions’ staff can operate in such a way to reduce transaction costs and to render communication smoother and flow of information faster. Communication and exchange of information could be made more effective thanks to the new ICTs (Information and Communication Technologies). It is critical to strengthen staff capacities in the State and Entity institutions, particularly the analytical and communication skills. In fact, since rural policy is strongly knowledge-based and multi-stakeholders, co-ordination and communication mechanisms play a key role in the design and implementation of place-based rural development policies. Communication should be developed horizontally, at the central, entity, regional and local levels, as well as vertically, across different government tiers (OECD, 2006). Motivation and incentives to public institutions’ staff can help in achieving this objective. That would allow to strengthen coordination and cooperation between them thus ensuring effective and efficient implementation of policies, programs, action plans and strategies and avoiding overlapping in responsibilities and activities.

A better coordination between involved institutions means not only to reduce institutions operating and transaction costs but also to manage effectively incentives and subsidies provided to farmers and rural dwellers and to avoid frauds, corruption and “*clientelism*”. That is true also in the case of the use of IPARD (Instrument for Pre-Accession on Rural Development) funds. Coordination is easier when all institutions have access to all information regarding agro-rural development that’s why it is crucial to speed up the establishment of information systems in agriculture. Moreover, financial support, from the EU and other international donors and cooperation agencies, should be provided not only for the establishment of these services but also for their operation and maintenance. A stronger partnership between Bosnian institutions dealing with rural development and those of the EU and its Member States can help to ensure a better cross-fertilization and exchange between them which can have positive impacts on their *modus operandi*.

Moreover, many of the solutions proposed by Bryden (2005) to address key coordination challenges and to achieve effective governance are suitable also in the case of BiH.

## Conclusions

Lack of a good coordination between actors dealing with agro-rural development policies decreases their effectiveness. Vertical coordination between State level institutions with Entity, regional and local ones, especially civil society organisations, is still particularly challenging in BiH. State and Entity governments should encourage local actors' participation in the design and implementation of place-based policies for rural development. That means that governmental and public institutions should redefine their role and devise new multi-level cooperation and coordination frameworks that emphasise power sharing between different governance levels and inter-dependence and partnership between a wide range of actors in agro-rural policy making. It goes without saying that the ease of vertical coordination between the different levels of governance also depends on the degree of horizontal coordination especially at the level of entities (RS and FBiH). Coordination between the Sector for Agriculture, Food, Forestry and Rural Development of the state MoFTER; the Ministry of Agriculture, Forestry and Water management of RS; the Federal Ministry of Agriculture, Water Management and Forestry (FBiH) and the Department for Agriculture, Forestry and Water Management of Brcko District (BD) is of a crucial importance. Coordination with other state and entity ministries and development agencies is also relevant. Civil society organisations, especially user ones, should be involved also in the design and evaluation and monitoring of agro-rural development policy.

In order to increase their impact, agro-rural development policies in BiH should be place-based, multi-sectoral, synergistic and designed and implemented through a good coordination between multilevel governance institutions. Although good governance is not sufficient on its own it is indispensable to sustain Bosnian rural territories development over the longer term. In the context of rural development, good governance should not be seen as a means to improve the living conditions of the rural communities by contributing to more appropriate and effective, and better coordinated services, based on participatory decisions, transparency and accountability. Dialogue and cooperation between state, entities, cantons, regions, municipalities and non-state actors is essential for promoting rural development. Local governance is to be put into the context of a wider process of institutional reforms of rural service systems encouraged by the EU and many other development agencies.

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## RURAL TOURISM AND FOOD PRODUCTION IN BOSNIA AND HERZEGOVINA

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### Abstract

Industrialized and developing countries increasingly develop rural tourism. It is particularly attractive for the population of highly urban areas and also because of the growing differentiation of lifestyles. Values that attract tourists in rural areas are: clean air, land, water, fresh food offer, peace and tranquility, cultural and historical heritage (monuments, folklore, country music) as well as other natural heritage (rivers, lakes, mountains).

Good examples of rural tourism are existing traditional ethno-villages, as well as newly developed ones. Existing tourist organizations offer visits to villages with daily or weekend arrangements, and full boarding arrangements. The share of foreign tourists in the total share of tourists in Bosnia and Herzegovina (B&H) is about 56%, and in the number of overnight stays is 52% and it differs by entities. According to the research, the total number of foreign tourists in BiH is about 1.4 million per year. There are typical products in Bosnia and Herzegovina that represent a basis for the development of tourism. Those are authentic sorts of fruit and vegetables, authentic breeds of cattle and poultry, as well as products received from them. Rural tourism can also be based on tourists' direct involvement in the countryside food production process, providing a concept of active vacation.

**Keywords:** Rural tourism, Forms, Authentic products, Development effects

### Introduction

Efforts to preserve natural environment, tradition and customs, to show and share them with a number people, leads to more intensive development of rural tourism, that is, activating rural areas for tourism purposes.

According to Hunziker and Krapf (2008), "*Tourism is the sum of the phenomena and relationships arising from the travel and stay of non-residents, insofar as they do not lead to permanent residence and are not connected with any earning activity.*" It is every journey that does not aim to perform an economic activity. A tourist is a person who travels at least 24 h.

Rural tourism is a trend both in Europe and world. It represents a form that includes a complexity of all activities and aspects of an integrated tourist product such as recreation in the countryside, enjoying environment and peace of rural areas, enjoying nature, national parks as well as nature parks, cultural tourism, tourism on rural households.

Rural tourism takes place in a rural area, outside the city. Residents of large cities are increasingly seeking rest and relaxation in some of the rural areas far from the city and the city noise.

Many villages provide an opportunity for development of rural tourism, within existing as well as those partly or completely abandoned. The concept of village could be defined as an area where the majority of population makes a living by a primary agricultural production and mostly breeding animals and growing plants. Natural heritage such as arable land, forests,

rivers, lakes and climatic conditions are usually the main factor that influences the development of a country.

Some definitions of rural tourism are related simply to tourism in the areas of low population density. Rural tourist destinations can be defined as areas that tourists visit in which the primary motive is enjoying the country atmosphere with variety of activities.

In the Strategy for the development of rural tourism in Southern Europe it is said that in the villages is being developed a special type of tourism which in English is called “*rural tourism*”. In practice a non-urban area could be called a “rural area”, which gives name for this type of tourism (Cetinski *et al.* 1995). Development of rural tourism is a necessity for ensuring the existence of rural areas. It is necessary to use all the comparative advantages that rural areas have. Also the development of rural tourism recently had a very significant and important tourism product in the domestic and international tourism market.

Rural tourism has a key role in the development and growth of economy, it also enables employment increase especially in rural areas, which includes young population and women. It also enables population migrations from urban areas to uninhabited and deserted places, especially of unemployed people in those places (OECD 2010). In general, the main functions of tourism are:

- contributes to employment increase of a large number of the population (directly or indirectly, through development of SME, engaged in treatment and processing of agricultural products, gathering forest fruit, herbs, healthy food production, development of craft, homecraft, and rural tourism),
- contributes to increasing and creating income,
- major source of foreign exchange income,
- contributes to increased revenues from taxes, fees, sales tax, tourist tax etc.

Attractiveness of rural tourism is assessed by the availability of healthy food, unpolluted air and water, healthy climate and the environment, peace and tranquility, preserved nature, preserved social and cultural heritage, restaurants, good infrastructure, picturesque region and others.

The greatest benefit from the development of rural tourism would have farms and the local economy.

According to the World Tourism Organisation (WTO), the concept of rural tourism is based on rich natural resources, rural heritage, rural lifestyle and rural activities. Natural resources include mountains, rivers, lakes, forests, and rural heritage includes traditional architecture, industrial heritage, history, castles, churches, villages. Rural life is related to entrepreneurship, local events, gastronomy, traditional music, rural activities related to horse riding, cycling, fishing, hiking and sports.

Production and distribution of food has importance in all forms of offer in rural tourism whether it is about participation of tourists in the harvest, sowing, harvesting fruit, accomodation on farms, gathering forest fruit, working on farms, horse riding, tasting products such as jam, honey and other products and beverages, milking cattle or visiting only for the consumption and supply of food and beverages. Also, food is a part of the offer of rural tourism within teaching of making products such as cheese, jam, wine and so on.

Besides, in the food offer it is also included shopping on farms or shops with farm products, buying local or regional products or handcrafted gifts.

### **Rural tourism as a specific type of tourist offer**

Rural tourism, agritourism, village tourism are often identified and imply the same term. A wide range of activities from rural supply can be successfully offered to tourists within rural tourism in Bosnia and Herzegovina. There are different definitions of rural tourism in different parts of the world but it always includes:

- a stay on the farm,
- buying products directly from the farm,
- picking fruit, feeding animals, on a farm.

Rural tourism include all the activities that bring visitors to farm or ranch that are mostly within village rural area. Tourist offer in the rural area could be expressed through other forms of tourism such as:

- agritourism,
- recreation and relaxation in nature,
- ecotourism,
- rural experience,
- cultural tourism: related to culture, archeology and other cultural content,
- other combined forms of tourism: tourist travels to rural areas and so on, as well as all services that complement accomodation – various events, festivals, outdoor recreation, production and sales of local souvenirs and agricultural products and so on.

The potential of B&H in different types of rural tourism is briefly described hereafter:

- Ecotourism includes types of tourism that support protection of natural resources, as well as maintenance of well-being and social value of local population (Anderson 1997). Ecotourism in Bosnia and Herzegovina, whose potentials are natural beauty, forests, rivers and lakes, represent also a quality base for the development of a unique tourist product on one area.

- Mountain tourism in Bosnia and Herzegovina: the country boasts beautiful mountains, some of which are already known ski resorts (such as olympic mountains of Bjelasnica, Jahorina, Vlasic, Igman, Trebevic). Excellent terrain, plenty of snow, suitable climate, slopes for all disciplines and gentle slopes, make mountains very attractive tourist destinations (interesting centers of mountain tourism open throughout the year and not only during winter season – Vlasic).

- Spa tourism in Bosnia and Herzegovina: there are several spas with various treatment potentials, that should be complemented with recreational components through investing in modernization of spas, introducing wellness and fitness programs (Ilidza spa near Sarajevo, Kiseljak, Tuzla, Fojnica, Srebrenica, Olovo, Teslic. This potential is favored by the abundance of springs of thermal-mineral water and radioactive water.

- Religious tourism in Bosnia and Herzegovina: churches, monasteries and mosques dating from the 16<sup>th</sup> century, and other places of worship of other confessions represent diverse and great cultural and historical richness that is only at the beginning of tourist valorization (brochures of Orthodox monasteries, places of worship on tourist auto maps, presentations on fairs and websites of tourist organizations, programs of visits to places of worship). Tourist organizations work on valorization and promotion of religious tourism. Among better known are Prusac, Jajce with numerous built capacities, cultural monuments of three cultures-religions (Islam, Orthodoxy and Catholicism).

- Marine tourism in Bosnia and Herzegovina: Neum Bosnian and Herzegovinian exit to the Adriatic Sea (Mostar, Ston Bay, Hutovo blato).

- Adventure tourism in Bosnia and Herzegovina is related to the rivers of Drina, Tara, Una, Vrbas with great potentials (World Rafting Championship held every year which is well

valorized in terms of tourism). There are also paragliding, mountaineering, cycling, as well as the possibility of development of increasingly popular canyoning.

- Hunting and fishing tourism in Bosnia and Herzegovina is oriented towards the domestic tourists. Most of the formerly well-attended hunting grounds attracted western clientele. By improvement of the infrastructure these areas will again become interesting to western tourists-hunters.

Rural experience is part of rural tourism where tourists live everyday rural life, and rural areas benefit through economic and other profits of tourist activities.

Any natural attraction is a potential tourist attraction that contributes to the development of tourism in Bosnia and Herzegovina.

As a main motive for directing tourists towards rural areas could be considered a desire to stay in a preserved natural environment and sense of freedom, authenticity and tradition, which together result in a feeling of relaxation and calmness.

It is important to point out that tourism is based on hospitality. Hospitality is a relation between guest and host or a practice of hosts being hospitable. In particular, it includes reception and entertainment of guests, visitors, or foreigners, but also respect for some codes and conventions. Hospitality also includes providing guests with desired attractions, special events, as well as other services for tourists. Population of Bosnia and Herzegovina need education in the area of hospitality and raising awareness level about the importance of hospitality in all forms of tourism and particularly in rural tourism.

#### **Food offer and perspectives of rural tourism in bosnia and herzegovina**

Bosnia and Herzegovina has great potentials for rural tourism development. The largest potentials are natural and cultural-historical heritage as well as habits and life of local population.

There are people who do not like or can't imagine living outside the city, but there is also a population that can't imagine living in urban areas. For them rural areas provide much larger benefits and enjoyment (peace and well-being), and therefore they gladly decide to spend life in the countryside.

In the present conditions, migration from village to town is decreasing, and it will continue to grow in the future. Namely, in rural areas there are various unresolved social and economical problems (reduced agricultural activity, high competition among local farmers in the same community).

Development of rural tourism in Bosnia and Herzegovina is based on rich natural heritage, unpolluted rivers, high, medium and low mountains, variety of flora and fauna, mild climate – a motivating factor for attracting foreign tourists and cultural and historical heritage, various forms of plant and animal communities, specific ecosystems, thermal, thermal-mineral and mineral waters, vicinity of the Adriatic Sea.

In the future ecological and biodynamical farms should be developed, which would be the foundation of sustainable development of ecological tourism, purchasing and reconstruction of old and abandoned farm-houses for enjoyment and disposition of both domestic and foreign tourists.

Population in Bosnia and Herzegovina lives predominantly in rural areas (60 to 70%), where also the poverty is most common. The most important activity is agriculture, secondary and poorly present industry, trade and crafts. Rural tourism has potential for decreasing poverty and improving quality of life in rural areas.

Data from the statistics of Bosnia and Herzegovina show that in 2011 74,585 tourists visited BiH, 1.5% more than in 2010. Number of overnight stays was very small (156,131, 0.2%). Development in tourism experienced growth but much slower than expected. Tourist



overnight stays of domestic tourists was 46.2%, and 53.8% of foreign tourists. Most overnight stays were realized by tourists from the region (Croatia: 13.6%, Serbia: 12.8%, Slovenia: 6.9%) rather than by tourists from other countries.

World Tourist Organization predicts that international tourism will continue to grow with an annual rate of 4% by 2020. Europe will be the most popular tourist destination, number of travels will grow in long-term period until 2020. According to data in Europe 23% of tourists annually choose rural tourism (Roberts and Hall 2001) and there is an estimated number of 200,000 registered service providers and more than 2,000 000 beds, with direct annual consumption of about 12 billion euro.

### **Natural resources and historical cultural heritage as a basis of the development of rural tourism**

B&H has huge natural potentials especially in villages in central Bosnia and in Herzegovina but the big problem is that there are not enough accomodation capacities. The lack of accomodation capacities, offer of domestic products and ignorance of local population of tourism prevent foreign tourists to visit more our villages.

Tourism in national parks and other protected areas and areas of perserved nature are potential tourist destinations and products in which BiH has exceptional conditions for a complex development of tourism (for example the National Park Sutjeska which is according to UNESCO one of the richest ecosystems and most beautiful parks of Europe). Among other parks there are the national park of Vranica, nature parks of Vlasic, Pogorelica-Bitovnja, and regional parks as Fojnika rijeka, Pliva, Gornji Vrbas (SBK2008).

International tourist demand is directed towards ecological-touristic destinations. It includes mountain tourism in a healthy and clean ecological environment. Among the protected objects of natural heritage stand out: waterfalls on Pliva in Jajce, waterfalls on Jaglenica river, Novi Travnik, Prokosko lake, Vranica in Fojnica, Gorge of Vrbas above Jajce, Tijesno gorge, gorge between Krupa on Vrbas, Stara Tisa in Travnik. Geographical location, natural resources, economic resources, tradition, experience have a great importance for BiH, especially from the economic point of view, representing an area with great opportunity for investments worth investing and doing business on, and seen as a field for good and mutual profit.

BiH has a variety of rural touristic offers. Also every rural household can develop special forms of tourist offer: accomodation of guests on their properties, offer of their own domestic products from the homecraft, tasting of domestic products (brandy, wine), organizing trips in the surroundings.

### **Food production within the development of rural tourism**

Food production is of strategic interest for BiH, which with a significant share in the structure of GDP (about 15%) increasingly enables development of rural areas and food security of the population. With rural tourism is connected the offer of organic food. People who come to the country want to feel the atmosphere, want to eat healthy food. To such places come middle-aged tourists who spend a lot. Rural tourism includes something authentic, a branded product, which BiH still doesn't have.

Typical products are the most important potential in the offer of rural tourism in BiH, particularly agricultural and food products, where a combination of natural factors and specific production methods guarantee the expected quality.

Significant authentic dairy products in BiH are sour milk, whey, buttermilk, cheeses (white cheese, zarica dry cheese, cheese from the bucket, Travnik/Vlasic cheese, cheese from milk), and flour products such as lepina, flat bread, as well as religious types of bread. Significant authentic fruit products are prune, plum jam, apple jam, apple must, cabbage from Semberija, strawberries from Celic, potatoes from Glamoc.

Improvement of production of typical authentic food products is a basis for development of village and rural tourism, expansion of the offer of agricultural and food products, and in the same time a precondition for decreasing poverty and improvement of life quality in rural areas.

### Conclusions

The main objectives of development of rural tourism are attracting foreign and domestic tourists, the return of the young people to the countryside from urban areas, development of organic food production in the rural areas.

Significance of rural tourism is in the added value of products, improvement of sustainability of agriculture and life in the rural areas which enables creating alternative income sources in non-agricultural sector.

For rural population extra income from rural tourism can contribute to the revival of lost activities in production of typical food products, authentic dishes, folk arts and handicrafts.

The main task of the development policy of B&H should be attracting tourists as well as good positioning on the market with adequate tourist resources and smooth running of tourism policy towards creating long-term strategic potentials.

Well created marketing policy could contribute to rural tourism to become one of the significant drivers of development in all regions of B&H.

Population of B&H needs education in the area of tourist disciplines, particularly in raising awareness of the importance of hospitality in all forms of tourism as well as in rural tourism.

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## ECONOMIC EFFECTS OF DRIED SOUR CHERRY PRODUCTION IN SERBIA

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### Abstract

The fruit processing in the Republic of Serbia is primarily focused on the production of juices, alcoholic beverages, compotes, aromas, jams and marmalades. The potential for dried fruit production is enormous, but minimally exploited. The sole exception is dried plum production, which is very important. Considering a constant increase in dried fruit consumption in the world, it is necessary to seriously take into account the possibilities for dried fruit production in Serbia.

In this paper, various aspects of the cost-effectiveness of dried sour cherry production are analysed. It is about combination of fruit drying technology (osmotic and convective). The research was conducted on a “smaller” dryer with the capacity of 450 kg of raw material per day, which is suitable for the production on family farms.

The raw material, i.e. fresh sour cherry (60.2%), poses a dominant factor in the cost structure. The labour costs were also significant (22.7%), whereas the energy share was surprisingly low (3.5%). The obtained retail price of 792 RSD (7.07 €/kg) is more competitive on the domestic market. This level of selling prices enables the profit of 1,406 € during 15 days of sour cherry production.

Previous research, which included a greater number of fruit species, indicate that the total profit during 135 days of effective dryer operation was approximately 15,200 €. The total investments, which are not high (approximately 30,900 €), are repaid in 1.82 years, which is very acceptable. Considering other success indicators (the efficiency ratio of 1.35 and the production accumulation rate of 26.0%), it is evident that this is a highly cost-effective business.

**Key words:** sour cherry drying, combined technology, profitability, comparative analysis

### Introduction

A sour cherry is one of the oldest fruit species that man has used since the ancient time. The origin of the sour cherry is the area from the Caspian Sea to Istanbul. As a adaptive fruit species, it can be cultivated up to 1,000 meters above the sea level, but its most appropriate fields are from 400 to 800 meters above the sea level. For centuries people had been breeding sour cherry varieties the most adaptable for the agroecological conditions in Serbia. By the grafting way of production, many native ecotypes of sour cherry have been introduced (the most popular is Obla inska variety).

Sour cherry is a perspective fruit species whose production is continuously increasing in the world as well as in our country. Distribution area of sour cherry is wide because this kind of fruit does not have specific requirements in terms of environmental conditions. However, despite the modest requirements in terms of natural conditions for production results, sour cherry market is scarce not only in Europe, but also in the whole world. Furthermore, important issue is providing a sufficient number of workers for the harvest. The world largest sour cherry producers are: USA, Russia, Iran, Poland and Germany. Production of sour cherry in Republic of Serbia in a few last years was on average 28-30 thousand tons and it represents

an important export product (Statistical Yearbook, 2010). Due to its quality, sour cherry from our country is very reputable on the European market.

Sour cherry as a fruit species has a great economic importance for the country by the high utility value of fruits, both as for fresh consumption and for further processing. Sour cherry production is not particularly demanding in terms of cultural practices and does not require high investment per unit area. In fact, compared to the most of other fruit species, it does not require more extensive pruning, it is relatively good resistant to pests and diseases, it becomes very early productive and fruitful regularly.

The Republic of Serbia has very favorable natural and climatic conditions for growing sour cherry. Sour cherry is a significant and promising fruit our country, especially from the point of export to the international market. It belongs to a group of high dainty fruit and has also significant nutritional, medicinal, dietary and technological values (Cerovic et al., 2005).

Sour cherry fruit has a high biological value due to the content of solids, pectin, proteins, tannins, carbohydrates, organic acids, minerals, vitamins and other beneficial substances. Besides fresh consumption, sour cherry is very suitable as a raw material for the manufacture of various products: syrup, juice, jam, marmalade, compote and for other variety of desserts. Sour cherry can be used for drying and deep freezing. It is also used for the production of liqueurs and cherry brandy, followed by using in confectionery industry for filling chocolates.

Processing of sour cherry by drying is not so much spread in our country. Research at the Faculty of Agriculture in Novi Sad has shown that sour cherry can be successfully dried by the mixed fruit drying technology. It remains to consider the economic viability of the drying sour cherry in the local conditions, which represents the main objective of the present study.

### **Material and methods**

Processing of sour cherry by drying can be applied only through the conventional procedure (conventional drying), which represents the most common way of drying. The sour cherry fruits can be also exposed to combined drying method, which consists of osmotic and convective drying. Osmotic drying is performing in sweet dilution of saccharose or of some other sugars. Unlikely to candying process, which is performing in saccharic dilutions and lasts up to several days, process of osmotic drying ends in several hours.

After osmotic drying, fruits is further drying by convective procedure until achieving required humidity. Applying osmotic drying method instead of candying has positive effects on conservation of fruit aromas and flavor. By the candying method, dominant product flavor comes from molasses.

The paper is based on profitability analysis of drying sour cherry by the application of the combined fruit drying method. Technological stages of drying, packaging and distribution are taken in consideration, but not production of fresh sour cherry. The combined fruit drying technology is based on original constructed devices in the Laboratory for Bio-systemic Engineering of the Agricultural Faculty in Novi Sad. The conducted laboratory research shows that this technology has positive effect on conservation of mechanical, visual and nutritional characteristics of the products, mass balance is favorable and rational energy consumption is accompanied by the possible usage of solar and biomass energy.

In the focus of the research are costs, achieved financial results and other productive-financial indicators of production of dried sour cherry. However, it is not possible to separately observe profitability of drying process of only one fruit species. By the fact that it is seasonal production and that during the whole year many different fruit species are used in drying process, it is important to take in consideration the profitability of total production, validity of investment project of construction and use of mini-plant for drying fruits respectively. Research is related to a "smaller" dryer with capacity of about 450 kg of raw

material per day, which is suitable for the family farms' production.

Aiming at the more detailed analysis and the clear presentation of achieved costs and financial results, calculation by technical activities (raw material preparation, osmotic drying, convective drying, packaging and selling) has been specially emphasized in the paper. Additional success indicators have been also introduced: margin coverage, income, pay-back time of invested funds, cost-effectiveness ratio and profitability ratio, sensitive analysis.

### **Results and discussion**

Considering profitability of future business activities, it is necessary to evaluate the total market potential and the amount of invested funds.

There are no precise data of sour cherry consumption in Republic of Serbia, but it is definitely on the modest level. Production and market demand for sour cherry are also on very low level in our country thanks to lack of habit of local consumers for this product. By the following increase of local consumption, it is obligatory to invest in marketing in order to create higher market demand. Higher possibilities of selling dried sour cherry would be firstly as a mixture with other fruit species and then as a stand-alone product. Undoubtedly, there is high potential for the growth and development of the domestic market, especially because of expected increase in living standard and by the fact of changing consumers' habits. Additionally, there are also significant opportunities for export.

Construction of small plant for drying fruit on family farm requires investments in purchasing dryer and equipment, building of new objects or reconstruction existing objects, building of cooling object, purchasing boilers for thermal energy needed for drying and the construction of the boiler room. There are also investments in infrastructure (roads, gas network, electricity), which occur to a greater or lesser extent. The total investment value of plant for drying fruits with capacity of 500 kg fresh fruit amounts 30,900 €. If there is suitable building in the commercial yard of the family farm, construction costs of the facility are reduced enough to carry out renovation (*Vukoje and Milic, 2011*).

Calculations are based on the following most important technical-technological and production-economic presuppositions:

- real daily capacity of dryer is 450 kg of fresh sour cherry, the plant is used effectively for 135 days per year, out of which 15 days are used for drying sour cherry (6,750 kg of fresh, i.e. 1,156 kg of dried sour cherry), while the rest of the days are used for drying other sorts of fruit (apricot, nectarine, peach, plum and apples), working in three shifts, it requires 5 workers;
- energy for osmotic drying is generated from electric energy, while for the convective drying it is generated from wheat straw, substitution of wheat straw by natural gas is also taken in consideration;
- investments in purchasing equipment and object, including cooling object, amount 30,900 € half of which is financed from credits, working capital is entirely financed from own resources;
- calculations are based on realistic market prices of inputs and final products in the period VI-XII 2012, all the prices are without VAT, free delivered (1€= 112 RSD) ;
- calculations are based on the laboratory determined expenditures of materials, labor and energy, and on the following mass balance:

**Table 1.** *The mass balance of drying cherries*

<b>Name</b>	<b>%</b>	<b>kg</b>
Dry halves	13.12%	59.0
Dry cubes	4.00%	18.0
Dried core	0.78%	3.5
Husk	3.14%	14.1
Usable waste*	3.66%	16.5
Unusable waste	1.97%	8.9
Evaporated water	73.33%	330.0
<b>Sum:</b>	<b>100%</b>	<b>450.0</b>

\* whole fruit that can be used for brandy etc.

Precise calculation requires cost monitoring by the technological production stages (preparation of raw materials, osmotic drying, convective drying and finalization). Direct costs are primarily calculated daily, then, on the basis of the established RSD exchange rate, calculations for 15-day sour cherry production are made (*tab. 2; graph. 1*). For calculating the general costs the procedure is inverted: the costs are determined on a yearly basis, and then divided by 135 days of planned effective operating of the dryer, to reach a daily amount.

**Table 2.** *Production calculation of dried cherry**(1€ = 112 RSD)*





Production of sour cherry requires daily costs of 29,890 RSD, which amounts 448,356 RSD (4,003 €) in the 15-day period. Regarding the overall costs, as expected, the costs of fresh sour cherry as the basic raw material are dominant with 60.2% (Fig. 1). Labor costs are also very important item (22.7%), primarily due to the relatively low level of automatization process. Fixed costs account for 8.8% of the overall production costs (depreciation and maintenance, general expenses and interest). General expenses include a proportionate share of overheads of family farm (telephone, fuel and travel expenses, insurance, fees and taxes for property, administrative services, potential sales costs, etc.)

*Fig. 1. Costs structure of dried cherry (%)*

The share of energy costs is very low (3.6%), which is mainly caused by using biomass (wheat straw) for convective drying. However, it is also very important to emphasize the relatively low cost of electricity (about 7.2 €cents / kWh). Logically, there is the issue of substitution of straw by natural gas, which is from the technological and organizational viewpoint considerably more suitable fuel. The equivalent amount of natural gas is about 67 m<sup>3</sup>/day, which, for the price of 40 RSD/m<sup>3</sup>, amounts 2,680 RSD/day. This increases energy cost for 277 €15 –day period, 2,490 €135-day period respectively. Beside that fact, their share remains acceptable with 6.7%. The cost of the dry half and dice is the same and amounts 388 RSD/kg (3.43 €/kg). When we calculate 20% trade margin and 20% VAT to the wholesale price of dried quarters of 550 RSD/kg, we get the retail price of 792 RSD/kg (7.07 € kg). This could be considered as competitive price for the domestic market.

The expected level of sales price ensures the profit of 1,406 € for the planned 15-day production of 1,156 kg of dried sour cherry. A farm can have significant additional benefit if employing two members of the family (40% of total manpower). In this case, the profit can be expressed by the income of the farm in amount of 1,771 €15-day period, whereas additional benefit amounts about 3,279 € per year (tab. 3). Cost-effectiveness coefficient (1.35) and profitability rate of dried sour cherry production (26.0%) also have very good values.

**Table 3.** *Additional indicators of success*

Since throughout a year some other fruit sorts are dried and since they have different levels of profitability, by the indicators of dried sour cherry production is not possible to make accurate projections of success on the annual level. Taking into account average profit of the most important fruit productions (apricot, pear, peach, quince and sour cherry) for which it has been calculated on average in amount of 15,209 €/year, by adding average annual depreciation (1,733 €/year), average financial flow amounts 16,942 €/year (Vukoje et al, 2010, 2011, 2012). By this assumption, the total investment is repaid for around 1.82 years, which is a highly acceptable period (*tab. 2*). The subsidizing of "smaller" construction for drying fruits from the state directly contributes to the shortening of this period.

### Conclusion

The analysis of economic parameters shows that dried sour cherry production is very profitable (profitability rate is 26.0%, cost efficiency 1.35). Moderate price fluctuations of fresh sour cherries and/or energy can not threaten the conclusion. The whole business project of building a mini plant for drying fruit on the family farm is also profitable. The relatively modest initial investment of around 30,900 €repays by about 1.82 years. The present national program of subsidizing the construction of new capacity in agriculture significantly cheapens the amount of investments. By the employment of family members, it possible to achieve an additional benefit in the form of salaries of about 3,280 € year.

Republic of Serbia possesses not only natural, but also considerable market potential for production of dried fruits. Beside the fact that imported dried fruits dominate on the domestic market, there are also significant opportunities for the export. The construction of small plants for drying fruits can be a good way for increasing income of family farms, reduction of unemployment and overall development of the countryside. Serious subsidy programs for building mini dryer as well as support for export products would significantly contribute to the development of this business.

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