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SUPPRESSION OF OAK POWDERY MILDEW THROUGH USE OF BIOFUNGICIDES

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Abstract

Serbia is involved in the International Co-operative Programme forest programme through its National Focal Centre. The condition of forests in Serbia has been monitored since 2003 during the vegetation period, at 131 sample plots. The occurrence of mass dieback in oak forests is another consequence of the presence of powdery mildew, which is caused by the pathogenic fungi *Microsphaera alphitoides* Griff. et Maubl. and which affects in particular new, young foliage susceptible to infections. Oak powdery mildew is a serious problem on seedlings in nurseries as well as on naturally and artificially introduced progeny. To date, the Republic of Serbia has registered no fungicides for suppression of pathogens in the forest ecosystems. In order to introduce proper use of new disease-fighting agents into a country, certain relevant principles, requirements and criteria prescribed by the Forest Stewardship Council . The experiments were set on penduculate oak seedlings in nurseries, where control of oak powdery mildew had been conducted through alternative protection measures by means of various dosages of AQ-10 biofungicide, with and without added polymer. The results of the research have demonstrated that AQ-10 biofungicide can be used as a part of integrated disease management programmes as an alternative, through application of several treatments during vegetation and combination with other active matters registered for these purposes, so as to curtail the use of standard fungicides for control of powdery mildews on oak seedlings in nurseries. The best results in suppression of oak powdery mildew were attained through use of AQ10 biofungicide with added polymer Nu Film-17.

Key words: *powdery mildew, sample plots, biofungicides*

Introduction

Consequence of the strong development of science and technology changes and disruption of the natural balance, which ultimately leads to the survival of some plant communities. However, with increasing risk increases the awareness of people that are at risk, and in this context qualitatively changing value systems of society as a whole, leading to the formation of ecological culture. Sustainable management of forest resources, policies and environment must be focused scientific approaches that enable long-term intensive monitoring of forest conditions on a large scale, which is one of the main goals of ICP (Cumming *et al*. 2001, Metzger & Oren, 2001, Nevenic e*t al*., 2009). To be in a country introduced the proper use of new products for diseases control, it is necessary to comply with any relevant principles, requirements and criteria, which are primarily related to measures of assessment and mitigation of risks, the list of hazardous and extremely hazardous pesticide, with the ability to application of alternative care, which are prescribed by the (FSC). To the management of forest resources were defined as viable, it must be primarily environmentally oriented, or adverse effects of management on the environment must be properly assessed and as small as possible (Sekulic, 2006). It is therefore against harmful organisms necessary to take measures for fighting that alternative chemicals within antipesticidne legislation. These measures include the use of biodegradable, non-chemical means, without the negative side-effects on non-target organisms without harmful effects on the environment.

Material and methods

The Republic of Serbia are included in the ICP program for forest owners, through its National Centre (NFC - National Focal Centre) for forest monitoring. The methods are described in the first Handbook as Visual Assessment of Crown Condition and in Submanual on Visual Assessment of Crown Condition on Intensive Monitoring Plots. Background and policy work are defined by the working group of the European Commission for Forestry (EFC) and the International Organization for Food and Agriculture (FAO). The Manual was redesigned in 2012 and provides harmonized data and a more flexible approach to monitoring the state of the crown, with better quality and more transparent. All parameters are described in the latest version of the Manual. They have been tested in several Europe countries or countries in North America, and the values of the parameters are continuously monitored under the control of the international Expert Panel. In Serbia, monitoring of the crown state is carried out during the growing season on the 131 sample plots. Oaks were on the 68 points. On this point were done a visual assessment of oak powdery mildew caused by fungi *Microsphaera alphitoides* Griff. et Maubl. (Syn. *Erysiphe alphitoides*). In Serbia it is presented almost everywhere where there are oak grown. A young, soft leaves are more susceptible to infection than mature. So that seedlings in nurseries and in the woods were more vulnerable than adult trees in the stands. Therefore, it was testing the efficacy of biofungicide AQ10 on young oak seedlings in two different nursery in Serbia (I - „Rogot“ – near Kragujevac and II - „Barosevac“ near Lazarevac).
In this study it was investigated the efficacy of biofungicide AQ-10 (the active component is *Ampelomyces quisqualis*, which attacks and destroys the fungi which causing powdery mildew). During the experimental tests biofungicide were used with the polymer Nu Film. This polimer were used in two different formulation Nu Film-17 and Nu Film-P. Polimer prepared a protective coating (film) and have a two-fold to prevent washout of the active ingredient of biofungicide and mitigate the realization of new infections of powdery mildew. For standard product it was used fungicide Sulphur SC (Table1).

Table1. Examined products and the dose/concentration of application

No	Product	Composition	Manufacturer	Mode of action	Dose/concentration
1.	AQ–10	***Ampelomyces quisqualis*** $5X10^9$ sporae/g	Bio Intrachem Italia	Biotrophic	30-50-70 g/ha
2	Nu Film-17	di-1-p-Menthene 96%	Miller- Chemical&Fertilizer Corporation, Hanover, Pensylvania	Encapsulated pesticide and protects it from various weather conditions	1-1.5 l/ha
3.	Nu Film-P	di-1-p-Menthene 50%	Miller- Chemical&Fertilizer Corporation, Hanover, Pensylvania	Encapsulated pesticide and protects it from various weather conditions	0.3-1 l/ha
4.	Sulphur SC	Elementary sulphur 810.50 g/l	Galenika - Belgrade, Zemun	Preventive	0.5%

Experiments were carried out on the instructions of methods PP 1/152 (2) (EPPO, 1997) in a randomized block design. The emergence and development of powdery mildew, followed by the first appearance of the disease and the development of the control treatment (when there is a clear difference between the control variants and variants treated with fungicide and biofungicide combined with a polymer). Estimation of secondary infection on the leaves was carried out on 100 leaves in four replicates per variant.

Results and discussion

Health status of oaks and monitoring of powdery mildew in Serbia

If we consider all oak species present throughout the study period, *Quercus pubescens* Willd. showed best results in terms of drying and chlorosis. Almost all the trees (from 92.3 to 100% of the trees) had index chlorosis 0 best state of health immediately after pubescent oak showed *Quercus cerris* L., who in category 0 chlorosis for the entire time of the study, with 71.8% and 97.0% of the trees.The third in the investigated area in Serbia is *Quercus farnetto* Ten. The highest percentage of *Q. farnetto* had chlorosis with index 0. Sessile oak - *Quercus petraea* (Matt). Liebl. The state of health comes at fourth place in the investigated area of the Republic of Serbia.The highest percentage of oak trees had chlorosis indexes 0 and 1 indicating the relatively healthy trees, but these percentages are lower than the previously mentioned species of oaks.

At the last place on health, when the parameters of observed chlorosis and drying, there is *Quercus robur* L. This species of oak had the highest percentage of trees found in chlorosis 0 (from 33.9% in 2004 to 83.3% , 2003), but the relatively large procent trees and in categories 1 and 2 and as high as 35.6% in the index 2, 2004, and there are plenty of trees in the index 3.

At the investigated oak trees infestation throughout the study period is generally very strong and is an average of 49.16% and a maximum of 79.3%. Intensity of infection has changed, so that the beginning of the study did not show the presence of powdery mildew, but the following year saw the emergence of a weak attack of 8.6%. Next, in 2005, recorded the culmination of infection or heaviest infestation, but during the study period infection gradually decreased, and in 2009 amounted to 51.8%.

Control of the oak powdery mildew with biofungicide

In Tables 2 and 3 were showed the data of the intensity of infection of oak on the research areas, the percentage of efficacy of the preparation in relation to the standard (Sulphur) and control variant. Based on the results shown in Table 2, the highest efficiency was obtained in the variant 3 which is used in the AQ-10 at a dose of application of 50 g/ha, with the addition of NuFilm-17 at a dose of 1.5 l/ha (2.35% of the intensity of the infection).

A good efficacy is achieved in the variants 2 and 6 (4.65% and 4.60%) in which the AQ-10 was used with polymer Nu Film-17 in a dose of 1.0 l/ha and 0.5% sulphur. Similar efficacy was achieved in 5 variants using AQ-10 with polymer Nu Film-P high application rates of 1.0 l / ha.

Table 2.The intensity of the attacks *M. alphitoides* and efficiency of the fungicide (I)

Number of variants	Fungicide	Conc./Dose (%), g, l/ha	Infection (%)	Efficiency (%)	Standard (%)
1.	**AQ-10**	50 g	7,15 a	64,07	83,33
2.	**AQ-10+Nu Film-17**	50 g+1 l/ha	4,65 a	76,63	99,67
3.	**AQ-10+Nu Film-17**	50 g+1,5 l/ha	2,35 a	88,19	114,71
4.	**AQ-10+Nu Film-P**	50 g+0,3 l/ha	6,35 a	68,09	88,56
5.	**AQ-10+Nu Film-P**	50 g+1 l/ha	5,70 a	71,36	92,81

6.	**Sulphur SC**	0,5%	4,60 a	76,88	100,00
7.	**Control**	-	19,90 b	0,00	0,00
	lsd 005		5,36		
	lsd 001		7,35		

The worst efficiency is shown in variant 1 where was used AQ-10 without the addition of the polymer (7.15% the intensity of the infection). In the control treatment in which protection was not carried out, the intensity of infection is 19.90%.
Statistical analysis of the obtained test results showed that there were no significant differences in all tested variants in comparison with the control variant. Based on the analysis of variance the difference between the variants were no statistically significant at the 95% , because of the $F_0 > F_{0,05}$. Also, comparative analysis (Duncan test, 1955) showed that there were no significant differences identified one homogeneous group. Based on the results shown in Table 3, the highest efficiency was obtained in the variants 6 and 10, or a variant in which is used a sulphur + NuFilm-17 and a variant in which the AQ-10 was used at a dose of application of 70 g/ha, with the addition of NuFilm-17 at a dose of 1.0 l/ha.

Table 3.The intensity of the attacks *M. alphitoides* and efficiencyof the fungicide (II)

Number of variants	**Fungicide**	**Conc./Dose (%), g, l/ha**	**Infection (%)**	**Efficiency (%)**	**Standard (%)**
1.	**AQ-10**	30 g	10,60 a	61,21	65,03
2.	**AQ-10**	50 g	12,42 a	54,53	57,93
3.	**AQ-10**	70 g	4,45 a	83,71	88,94
4.	**AQ-10 +Nu Film-17**	30 g + 1,0 l/ha	9,13 a	66,61	70,76
5.	**AQ-10 +Nu Film-17**	50 g + 1,0 l/ha	10,55 a	61,39	65,22
6.	**AQ-10 +Nu Film-17**	70 g + 1,0 l/ha	3,28 a	88,01	93,51
7.	**AQ-10 +Nu Film-P**	30 g +1,5 l/ha	12,40 a	54,62	58,03
8.	**AQ-10 +Nu Film-P**	50 g +1,5 l/ha	6,75 a	75,30	80,00
9.	**AQ-10 +Nu Film-P**	70 g +1,5 l/ha	4,25 a	84,45	89,72
10.	**Sulphur SC+Nu Film-17**	0,5%+1,0 l/ha	0,98 a	96,43	102,45
11.	**Sulphur**	0,5%	1,61 a	94,13	100,00
12.	**Control**	-	27,33 b	0,00	0,00
	lsd 005	10,34			
	lsd 001	14,61			

Slightly higher values of infection, but also a high degree of efficiency showed a variant of 11 which was used only sulphur (intensity of infection 1.61%).A good efficiency is achieved in variants 8 and 9 (6.75% and 4.25%) were AQ -10 (50 g and 70g) were used with the addition of NuFilm-P at a dose of 1.5 l/ha. Similar efficacy was achieved in 3 variants using

high concentration AQ-10 (70 g) without surfactant additives (intensity of infection of 4.45%).
Statistical analysis of the test results showed that there are no the significant differences in all studied variants compared with the control. There were no differences between variants 10 and 11, in which the fungicide were used with or without the addition of polymer.
Between the mean values of control and AQ-10 application doses as well as for all other combinations, there is a statistically significant difference at the probability of 99%.
In the first investigated the site, we applied a comparative analysis (Duncan test, 1955), in order to determine significant differences were identified 3 homogeneous groups, with statistically significant differences at 99%, which is consistent with the groups already explained variance analysis.
Low and high air temperatures (11 and 30°C) directly influenced the reduction of infection, which at that time was 10 and 32% of the total number of tested plants. Very high infection rate of seedlings was observed at temperatures of 17 to 21°C and at a relative air humidity of 85 to 100% (infection in this period was 51 to 63% of the tested plants.
In the literature to the many authors in different climatic regions confirmed and proved that the intensity of powdery mildew infection in various plant species directly dependent on environmental conditions, especially temperature and air humidity.
Comparative analysis of effects of temperature and air humidity on the occurrence of secondary infections and the spread of powdery mildew infection and the results of other authors (Kothari & Verma, 1972; Whipps & Budge, 2000; Guzman-Plazola et al., 2003) shows that high relative humidity reduces the intensity of the infection, which may aid in the control of this pathogen in the future.Temperature of 30°C and are more detrimental to the development of pathogens. The growth of the fungus is significantly higher at 20°C than at 25°C. High levels of relative humidity (80-90%) are favorable for the development of the pathogen in the short term, but prolonged exposure to these conditions leads to the limitations of the infection.
This means that the number of treatments is not crucial for achieving high efficiency and bioproducts. If the treatment is performed at an appropriate time, with fewer treatments and a lower dose of bioproducts achieve the same efficiency as well as the large number of treatments, and the higher doses of bioproducts, which is of course very important from the economic point of view, and this experience can be applied to control powdery mildew bioproducts in nurseries.

Conclusions

Results of testing of bioproducts AQ-10 showed good efficacy in controlling powdery mildew of oak in nurseries in Serbia, using several treatments during the growing season and the combination with other active ingredients registered for this purpose. The best results in the suppression of powdery mildew were achieved with oak embodiments in which the biofungicide AQ-10 was used in higher doses (50 and 70 g/ha) with the addition of the polymer film Nu Film-17 at doses from 1.0 to 1.5 l/ha.
The number of treatments is not crucial for achieving high efficiency and bioproducts. That is, if the treatment is performed at an appropriate time, with fewer treatments and a lower dose of bioproducts achieve the same efficiency as well as the large number of treatments, and the higher doses of bioproducts, which is very significant from an economic viewpoint. Properly administered, timely and professional use by selecting the appropriate preparation, provide the rationalization of the use of pesticides, as well as the reduction of the treated area. In accordance with the actual capabilities (in the case of limited possibilities of mechanical measures and the lack of labor force), use alternative solutions to protect forests or perform the combined use of pesticides and alternative methods.Within repressive measures, the

development and introduction of alternative methods of forest protection against harmful organisms is done in order to find suitable alternative products and methods of protection, in order to overcome the problem of exclusion of unwanted pesticides. Therefore, it is necessary to support academic institutions in research aimed at finding alternative methods and pesticides less harmful impact on the environment and biodiversity in forest ecosystems.

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