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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 epiphytoties, 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 $R_0$-$R_{11}$ 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) 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).

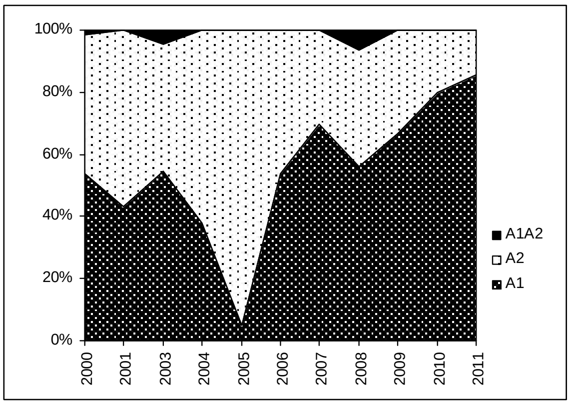



Fig. 1. Dynamics of changes in the ratio of different mating types in the *Ph. infestans* population of the Moscow region in 2000-2011.

**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).

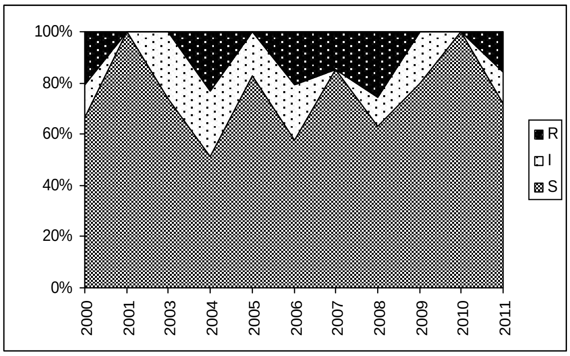
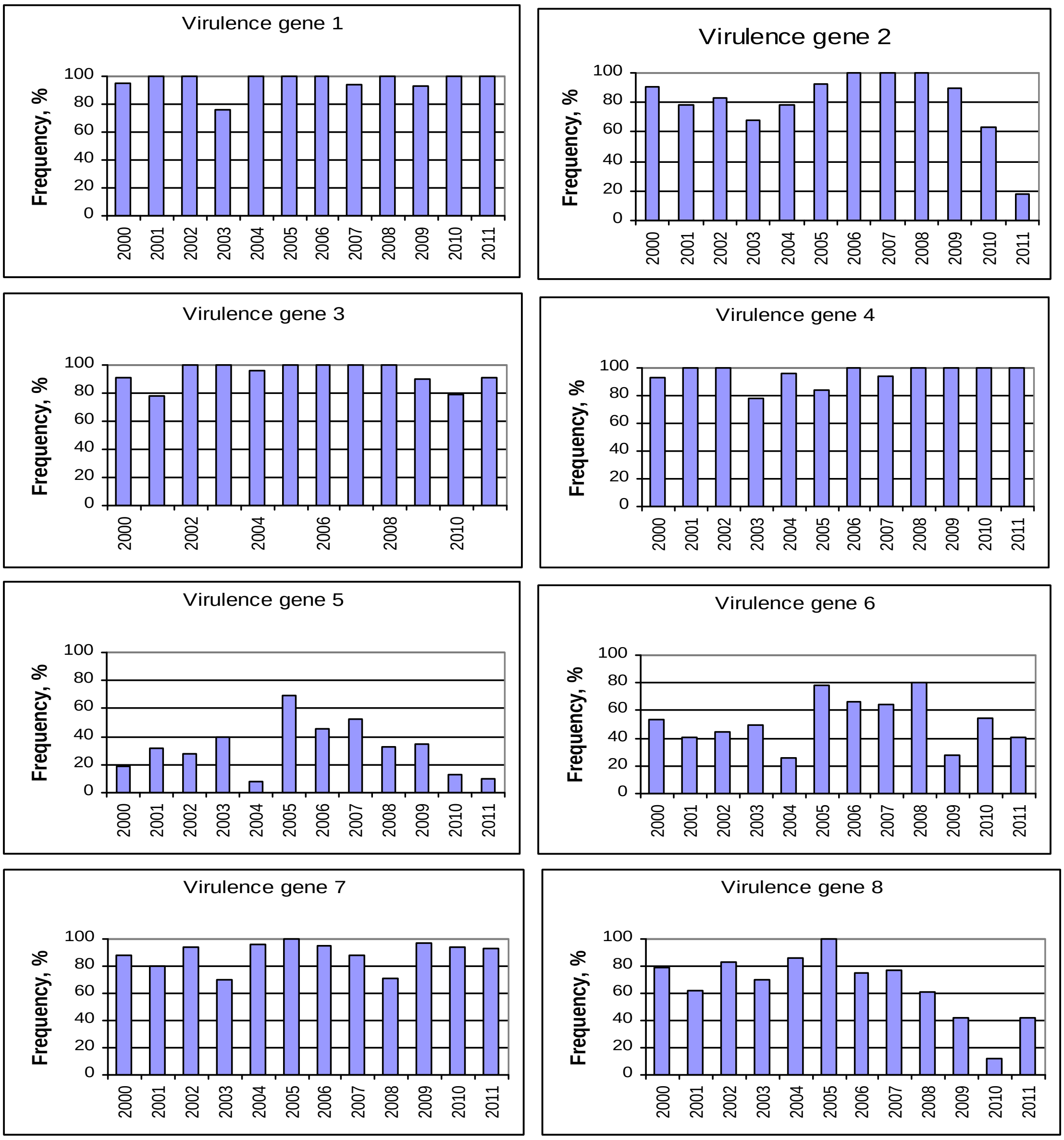



Fig. 2. Dynamics of changes in the metalaxyl sensitivity of the *Ph. infestans* population of the Moscow region in 2000-2011.

**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.



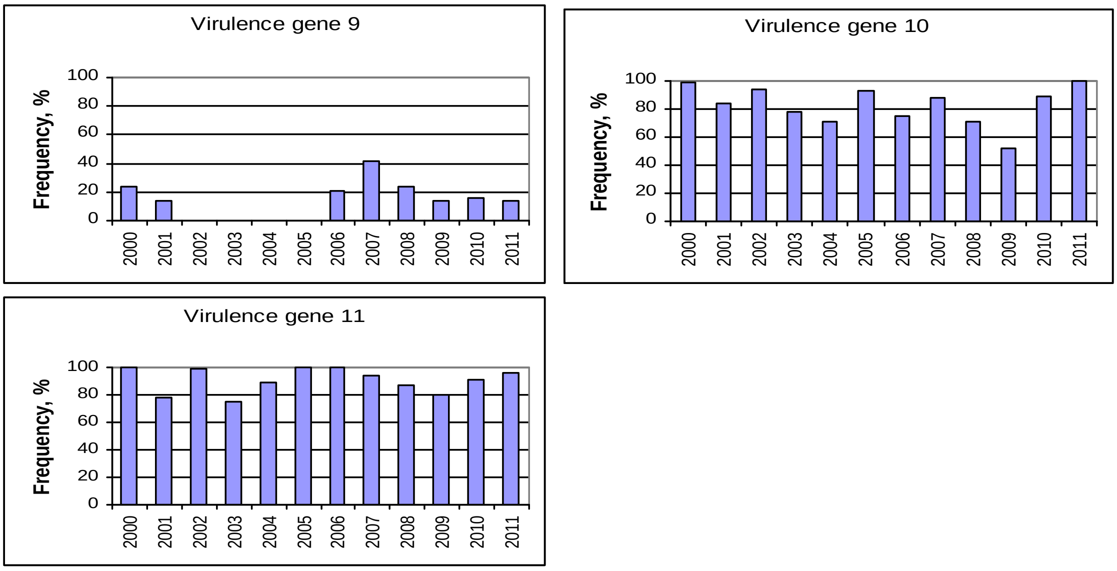



Fig. 3. Changes in the frequencies of individual virulence genes in the Moscow *Ph. infestans* population in 2000-2011.

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 betwen potato planting on the same field should not be less than 3-4 years.

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