Long-term study of the *Phytophthora infestans* population from the Moscow region of Russia (2000-2011)

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Abstract
The study presents results of a long-term monitoring of the *P. infestans* population in the Moscow region (2000-2011). A total of 1586 isolates were assessed using such phenotypic and genotypic markers as the mating type, metalaxyl resistance, two allozyme loci of peptidase, and a mitochondrial DNA haplotype. In addition, virulence patterns were determined. During all examined period, the total population diversity level remained high and both A1 and A2 mating types were observed. The population was presented mainly by complex races and included all 11 virulence genes. The diversity of „tomato“ subpopulation was lower than that of the „potato“ one concerning the most part of the used markers.

Key words: *Phytophthora infestans*, population, metalaxyl resistance, mating type, virulence

Introduction
Potato is one of the most important crops in Russia. The average annual consumption of potato per a person in Russia makes 120-130 kg, i.e. this crop remains a „second bread“ for many Russian people and significantly influences on the food safety of Russia, which now takes the second place in the world potato production (about 37 mln. tons/year).

Potato late blight, caused by the oomycete *Phytophthora infestans* (Mont.) De Bary, is the most devastating potato (and tomato) disease that is able to twice reduce the crop productivity during epiphytotes. In addition, this pathogen infects tubers, influencing on its storage quality; as a result, total yield losses, caused by this pathogen, can reach 60%.

In eighties, a sharp increase in the late blight severity was observed in Europe. During 1980-1985 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 the „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 *P. 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 long-term monitoring of changes in the *P. infestans* population of the Moscow region, which represents one of the largest potato-growing regions of Russia and the largest importer of a potato seed material, and the assessment of possible differences between the pathogen subpopulations, collected from different host plants (tomato and potato).
Material and methods

*P. infestans* isolates were collected during a period of 2000-2011 from commercial 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. The total number of *P. infestans* isolates, collected during this period, made 1586, including 1097 „potato“ and 489 „tomato“ isolates. All isolates were analyzed using common phenotypic markers (mating type, virulence pattern, and metalaxyl sensitivity), and a subset of 684 isolates was also analyzed using some of the common genotypic markers (two allozyme loci of peptidase and a mitochondrial DNA haplotype).

**Virulence.** To study the virulence of isolates, we used a set of differentiator potato cultivars, obtained from the International Potato Center (CIP, Peru) and containing 22 genotypes, including all known resistance genes in different combinations. We also used the test set, containing R$_0$-R$_{11}$ genotypes and obtained from the Institute of Plant Cultivation and Acclimatization (IHAR, Poland). The analysis was carried out under laboratory conditions using detached potato leaves as described in our earlier study (Statsyuk et al., 2010).

**Mating type** was tested by the growing isolates on rye agar with the known reference strains of the A1 and A2 mating types as described earlier (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 (Cohen and Reuveni, 1983). Depending on the obtained results, isolates were considered as sensitive (S), intermediate (I), or resistant (R).

**Allozyme analysis.** Genotypes at two peptidase loci (PEP1 and PEP2) were analyzed by a cellulose acetate gel electrophoresis using a standard procedure (Hebert and Beaton, 1993) with some modifications (Elansky and Smirnov, 2003).

**Mitochondrial DNA haplotype identification** was carried out according to the common procedure (Griffith and Shaw, 1998).

**Results and discussion**

**Virulence.** According to the obtained results (data not shown), the dynamics of changes in the frequency of individual virulence genes in the „potato“ (*P*) subpopulation can be described in the following way. The frequencies of the virulence genes 1, 3, 4, 7, and 11 remained at the stable level for the whole studied period, varying within the range of 8.6-14.2%. The frequencies of the genes 10 and 2 remained at about the same level as those of the above-mentioned genes; however, in recent years the frequency of the gene 2 began to sharply decrease (from 12.5 (2009) to 2.5% (2011)), whereas the frequency of the gene 10 increased from 7.2 (2009) to 14.2% (2011). The frequency of the gene 8 remained intermediate (8.3-10.9%) until 2005 and then began to gradually decrease up to 1.7% in 2010; in 2011 its frequency increased to 6%. The group of rare virulence genes includes genes 5, 6, and 9. The frequencies of the genes 5 and 6 stably remained at the low (1.4-7.6%) and low-intermediate (3.9-9.7%) levels, respectively; the frequency of the gene 5 gradually decreased since 2005 up to 1.4% in 2011. The gene 9, which has not been revealed in the Moscow *P. infestans* population before twentieth, stably presents in the population since 2006, though its frequency remains low (1.8-4.6%).

In general, *P* subpopulation was presented mainly by complex races, including from 5 to 11 virulence genes; the fraction of such complex races made 50-70%. The most complex race, including all 11 virulence genes, was observed in 2008-2011.

In the case of the tomato (*T*) subpopulation, the data we have cover only last three years (2009-2011). According to these data, the most frequent genes in this subpopulation are the genes 1-4, 7, and 11; they frequencies vary within the range of 9.9-18.6%. The frequency
of the genes 8 and 10 is low; finally, the rare genes 5, 6, and 9 were not revealed at all. Thus, the number of virulence genes in the $T$ subpopulation is less than in the $P$ subpopulation that determines the less diversity of this subpopulation.

**Mating type.** The results of the mating type analysis are shown in Graph 1 separately for each subpopulation. Both subpopulations include isolates of both A1 and A2 mating types; in some years the presence of A1A2 isolates, able to form oospores with the isolates of both A1 and A2 types, was also revealed. Therefore, the possibility of the sexual process within the pathogen population of the Moscow region remained rather high during the all analyzed period. At the same time, there is a difference between the $P$ and $T$ subpopulations concerning the dynamics of A1 and A2 frequencies. In the case of the $P$ subpopulation, the frequency of the „new“ A2 mating type reached the peak value (95%) in 2005 and then gradually decreased up to now (14.3% in 2011). In the case of the $T$ subpopulation, the A1 mating type dominated almost all this period; however, since 2007 the frequency of the A2 type increased up to 100% in 2010. Last year the A1 type frequency started to increase again, reaching 22.2%.

The trend to the gradual increase in the frequency of the A2 isolates in the first half of twentieth agrees with our earlier data, obtained for the same population in 1997-1998 (Elansky et al., 2001), when the average frequency of the A2 isolates in $P$ and $T$ subpopulations made 28 and 12%, respectively. Thus, since the first detection of the A2 isolates in Russia in 1985 (Vorobyeva et al., 1991) their frequency in the studied $P$ subpopulation continued to grow until 2005; then the A2 type started to lose its positions. In the case of the $T$ subpopulation, the frequency of the A2 mating type increased up to 2010; probably, the back process has already started.

Graph 1. Time changes in the proportion of different mating types in „potato“ (left) and „tomato“ (right) subpopulations of *P. infestans* from the Moscow region.

**Metalaxyl sensitivity.** The results of the metalaxyl sensitivity monitoring are shown in Graph 2. During the whole examined period, both subpopulations were represented mainly by sensitive isolates; in general, the percentage of resistant and intermediate isolates is slightly higher in the $P$ subpopulation that is probably explained by a more frequent use of fungicides on potato fields.

**Allozyme analysis.** The results of the allozyme analysis are shown in Graph 3. In the case of the PEP1 locus, the 100/100 genotype dominated in both subpopulations for the whole studied period. The presence of the 92/92 genotype was revealed only in the $P$ subpopulation (2008). In the case of PEP2 locus, the genetic diversity level was higher. Both subpopulations included all three observed genotypes. The genotype 100/100 was dominant in the $P$ population, whereas in the $T$ population its frequency was comparable with that of the genotype 100/112. The genotype 112/112 was rare in both subpopulations.
No any clear dynamics was revealed for these two markers.

Graph 2. Dynamics of changes in the metalaxyl sensitivity of „potato“ (left) and „tomato“ (right) subpopulations of P. infestans from the Moscow region.

Graph 3. Frequencies of PEP1 (top) and PEP2 (bottom) genotypes in the „potato“ (left side) and „tomato“ (right side) subpopulations of P. infestans from the Moscow region.

**Analysis of mitochondrial DNA haplotype.** The results of the mitochondrial DNA analysis are shown in Table 1.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>„Potato“ subpopulation</th>
<th>„Tomato“ subpopulation</th>
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<tbody>
<tr>
<td>Ia</td>
<td>65</td>
<td>63</td>
</tr>
<tr>
<td>IIa</td>
<td>35</td>
<td>37</td>
</tr>
<tr>
<td>I</td>
<td>50</td>
<td>33</td>
</tr>
<tr>
<td>II</td>
<td>50</td>
<td>67</td>
</tr>
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Only two of four possible haplotypes (Ia and IIa) were observed during the studied period.
The haplotype Ib, typical for the “old” *P. infestans* populations was not observed in Russia since 1993, whereas the haplotype IIb, typical for American populations, has never observed in Russia. The haplotype Ia dominated in both subpopulations in the most of the studied years; in recent years both subpopulations are represented by only this haplotype.

**Conclusions**

The performed analysis allows us to conclude that this population still remains very complex and diverse. During this period, a new virulence gene appeared in the „potato“ subpopulation, and now it includes all 11 virulence genes and consists mainly of complex races that provides its high aggressiveness level. The presence of both A1 and A2 mating types in the population provides a high possibility of a sexual process and the corresponding increase in the recombination frequencies and the generation of oosporas, able to overwinter on plant debris in the soil. The diversity of „tomato“ subpopulation is lower than that of the „potato“ subpopulation concerning the most part of the markers used.

**Acknowledgements**

This study was partially supported by the International Science and Technology Center (project no. 3714 „DNA markers of potato genes for late blight resistance“).

**References**


