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Characteristics of the *Phytophthora infestans* population in Russia

N.V. STATSYUK[1], M.A. KUZNETSOVA[1], I.N. KOZLOVSKAYA[1], B.E. KOZLOVSKY[1], S.N. ELANSKY[2], E.V. MOROZOVA[1], E.V. VALEVA[1], AND A.V. FILIPPOV[1]

[1]All-Russian Research Institute of Phytopathology, VNIIF,
Bolshie Vyazemy, Moscow region, 143050 Russia; e-mail: natafg@rambler.ru
[2] Department of Algology and Mycology, Biological Faculty,
Moscow State University, Vorob'evy Gory, Moscow, 119992 Russia

SUMMARY
A collection of 434 *Phytophthora infestans* isolates, obtained during 2007-2009 from potato and tomato fields of different parts of European Russia, has been assessed for several phenotypic and genotypic markers, including mtDNA haplotype, Pep1 and Pep2 loci, mating type, metalaxyl sensitivity, and the virulence. The comparison of phenotypic and genotypic characteristics of *P. infestans* population of the Moscow region in 2008-2009 with the similar data, obtained in 1997-1998, has shown significant changes occurred within a 10-year period: (1) the frequency ratio of Ia and IIa MtDNA haplotypes has shifted towards the predominance of the Ia type, (2) the percent of strains belonged to the A2 mating type has increased, (3) the virulence gene 9 has appeared in the current population, which now contains all 11 virulence genes, and (4) the percent of metalaxyl-susceptible strains has significantly decreased from 78 to 58%. The analysis of the studied populations has shown that the Leningrad, Astrakhan, and Nizhni Novgorod populations are rather uniform, whereas the Moscow, Mariy El and Kostroma populations are characterized by a high diversity. The Astrakhan "tomato" population has a unique pattern of virulence gene frequencies and seems to be monoclonal.

KEYWORDS
late blight, potato, tomato, population characteristics

INTRODUCTION

Phytophthora infestans, the causal agent of the late blight disease of potato and tomato, is the most damaging microbial pathogen of these crops world-wide, including Russia and Eastern Europe. In the XIX century this pathogen caused significant potato yield losses in the Northern Europe that resulted in the Irish Potato Famine. In the last decades, new agrotechnical and plant pest control methods, such as the use of certified seeds, breeding programs, crop rotation, and highly-effective fungicides, significantly decreased potato yield losses caused by the late blight. At the same time, due to a high pathogen variability and a possible introduction of new strains by a potato shipment from

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central Mexico to Europe, the European population of *P. infestans* has undergone significant changes [1-2]. The "old" population was represented by the A1 mating type and Ib mitochondrial (Mt) DNA haplotype, whereas the "new" population consisted of the isolates of both A1 and A2 mating types and Ia and IIa MtDNA haplotypes [3-7]. Among other changes in *P. infestans* population traits, one should also mention an increase in the metalaxyl resistance that decreased the effect of the use of popular metalaxyl-based fungicides on the late blight control [8].

The interaction between strains of different mating types can cause the sexual reproduction of *P. infestans,* increasing the genetic diversity of the progeny; this can possibly result in an increase in the virulence and fungicide resistance of newly developed strains.

In our previous studies we analyzed the phenotypic and genotypic characteristics of *P. infestans* populations from the Moscow region, Siberia, and Far East [9]. In this study we present the data, obtained for the Moscow region (2008-2009) and 5 new regions, including North Western Russia and several regions of the Eastern part of European Russia.

MATERIALS AND METHODS

P. infestans isolates were collected from commercial potato and tomato fields, located in the following regions of the European Russia (Fig. 1): Leningrad (21 isolate), Moscow (100 isolates, including 20 from tomato plants), Nizhni Novgorod (13 isolates), Astrakhan (31 isolates from tomato plants), Kostroma (105 isolates), Smolensk (49 isolates), and the Mariy El Republic (115 isolates, including 93 from tomato plants). The total number of the studied isolates was 434.

Allozyme analysis. Genotypes at two peptidase loci (Pep1 and Pep2) were analyzed using a cellulose acetate gel electrophoresis according to [10] with some modifications [11]. The genetic diversity for the Pep1 locus in Russian *P. infestans* populations is significantly lower than that of the Pep2 locus, represented by two alleles (100 and 112) and stained simultaneously with the Pep1 locus (Fig. 2), so we analyzed both these markers.

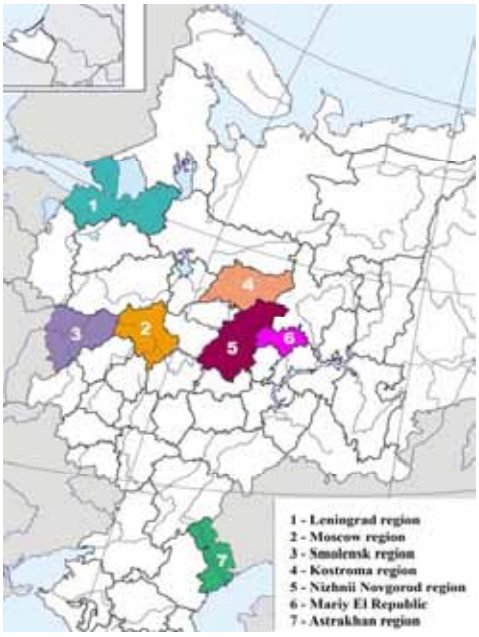

Fig. 1. *Locations of sampling sites.*

The mtDNA haplotype identification was carried out according to [12] with some modifications.

T*he mating type* was tested by the growing isolates on rye agar with the known reference strains of the A1 and A2 mating types. Agar blocks with the studied and reference strains were placed by pairs into Petri dishes, containing rye-vegetable agar, at a distance of 4-5 cm from each other. The Petri dishes were incubated in the dark at 18°C or 14 days, and then we microscoped the place of a hypha contact between the strains to determine the presence or absence of oospores. If the studied isolate generated oospores only with the A2 isolate, it was referred to the A1 type. If the isolate generated oospores only with A1 isolate, then it was referred to the A2 type. If the isolate generated oospores with both reference strains, then it was referred to A1A2 type.

Metalaxyl sensitivity. The sensitivity of isolates to metalaxyl-containing fungicides was determined by the inoculation of fungicide-treated tuber discs [13] or fungicide-containing

medium [14] with the tested isolates at different fungicide concentrations. According to the obtained results, isolates were considered as sensitive (S), moderately resistant (MR), or resistant (R).

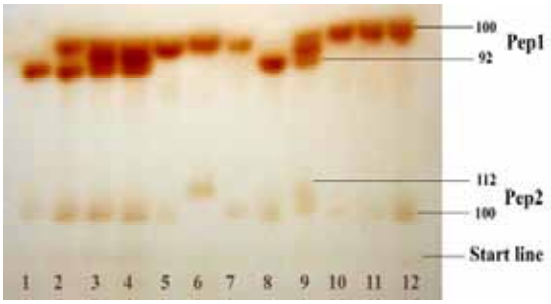

Fig. 2. *Cellulose acetate plate showing typical allozyme banding patterns of two peptidase loci for P. infestans isolates.* **Pep1 locus***: 92/92 (lanes 1 and 8); 100/100 (lanes 5-7, 10-12); 92/100 (lanes 3, 4, 9).* **Pep2 locus***: 100/100 (lanes 1-5, 7, 8, 10-12); 112/112 (lane 6); 100/112 (lane 9).*

Virulence. To study the virulence of isolates, we used a set of differentiator 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. The leaves were placed into a wet chamber, representing a frame (30x40 cm) with the bottom made of a metal gause. The chamber was covered with a glass sheet. To inoculate the leaves, we used a 4-5-day culture of *P. infestans*, grown on the tuber slices of a susceptible potato cultivar, or 10-12-day culture, grown on nutrient medium. A pathogen suspension was prepared in a concentration, corresponding to 10-15 conidia in a microscopic field at 100x magnification. Tubes with the suspension (5 ml) were placed into a refrigerator (6-7°C) to initiate the release of zoospores. The lower surface of each leaf was inoculated by 2 drops of suspension using a micropipette; after 24 h the drops were shaken off, and the leaves were turned upside down. The leaves were incubated at 18-20°C. The level of the disease development on the leaves was determined after 4-6 days of incubation. The presence of the fruiting testified a compatible reaction and was designated by a "+"; the absence of any fruiting was designated as "-".

RESULTS AND DISCUSSION

Allozyme analysis.

The results of the allozyme analysis for Pep1 and Pep2 loci are shown in Fig. 3.

In all populations the predominant genotype of the Pep1 locus was 100/100; all three "tomato" populations were represented by only this genotype. The presence of all three possible variants (92/92, 92/100, and 100/100) was revealed only in the Moscow "potato" population, like in the case of our previous study [9].

In the case of Pep2 locus, the genetic diversity was higher. All three possible variants were revealed in 4 populations at different proportions. Again, the most frequent genotype was 100/100, excepting the Leningrad population, represented by the only 112/112 genotype, Smolensk population, represented mainly by 100/112 genotype, and Kostroma population, where the 112/112 genotype predominated. It is interesting that the 100/100 genotype was predominant for all three tomato populations (100% for Astrakhan and Moscow regions and 47.8% for the Mariy El Republic).

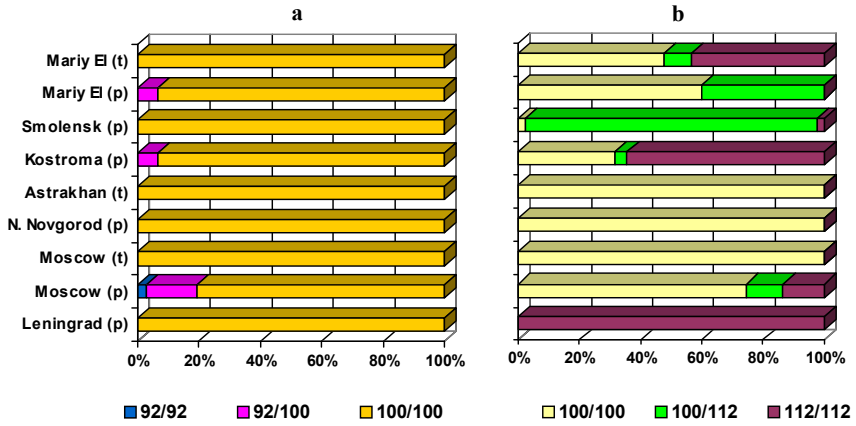

Fig. 3. *Frequency of Pep1 (a) and Pep2 (b) genotypes of P. infestans samples from different regions of European Russia; (p) and (t) mean "potato" and "tomato" populations, respectively.*

Analysis of mitochondrial DNA haplotype

The results of the mtDNA analysis are shown in Fig. 4.

We revealed only two mtDNA haplotypes (Ia and IIa). The Ia genotype was predominant for all populations, excepting the Mariy El "tomato" population; two populations (Leningrad and Astrakhan "tomato") were presented by only this genotype.

In the case of the Moscow region, some changes in the ratio of these MtDNA haplotypes were registered comparing to the previous data, obtained in 1997-1998 [9]. According to the earlier study, the Ia : IIa ratio was about 36 : 64, whereas the recent data show a tendency to the increase in the Ia percentage (55 : 45).

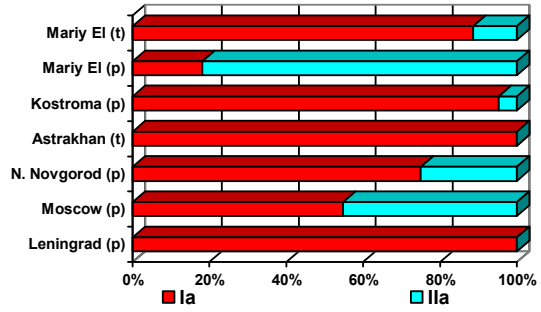

Fig. 4. *Mitochondrial DNA haplotypes of P. infestans samples from different regions of European Russia; (p) and (t) mean "potato" and "tomato" populations, respectively.*

Mating type

Mating type frequencies, determined for all studied populations, are shown in Table 1.

The A1 mating type predominated in most of the examined populations; in the case of Leningrad (potato) and Astrakhan (tomato) populations, it was the only variant revealed. The A2 mating type prevailed in the Nizhnii Novgorod, Smolensk, and Mariy El (potato) populations (100, 94.4, and 64.3 percents, respectively). Populations from the Moscow and Kostroma regions included a small percent of A1A2 strains, able to generate oospores with both reference strains.

Comparing the data obtained for the Moscow region in 1997-1998 [9] and 2008-2009, one can

note that the A1:A2 ratio in both potato and tomato population of *P. infestans* changed from 72:28 (potato) and 88:12 (tomato) to 61:35.4 and 65:35, respectively, i.e. the percent of the A2 strains increased.

Table 1. *Occurrence of A1 and A2 mating types in Russian P. infestans populations*

Region	Mating type, %		
	A1	A2	A1A2
Leningrad (potato)	100	0	0
Moscow (potato)	61	35.4	3.6
Moscow (tomato)	65	35	0
Nizhni Novgorod	0	100	0
Astrakhan (tomato)	100	0	0
Kostroma (potato)	80.1	19.4	0.5
Smolensk (potato)	5.6	94.4	0
Mariy El (potato)	35.7	64.3	0
Mariy El (tomato)	77.8	22.2	0

Virulence

The results of our virulence study are shown in Fig. 5.

According to our data, populations from the Kostroma and Leningrad regions were similar to each other: the frequencies of single genes were about the same, and both populations did not include the gene 9. Both populations have similar values of the factor of virulence (FV). The most frequent races in both populations included 8-10 virulence genes.

Both tomato and potato populations of *P. infestans* from the Mariy El Republic were represented by complex races and had the similar structure. The most frequent races were 1.2.3.4.6.7.8.10.11 (28.2%) and 1.2.3.4.5.6.7.8.10.11 (50%). The total percent of these two races in these two population was 78.2%.

The Moscow population included all virulence genes and was presented mainly by complex races, containing 7-11 genes (including gene 9, which was absent in the most of the studied populations). The most frequent races were 1.3.4.7.8.10.11; 1.2.3.4.6.7.8.11; 1.2.3.4.6.7.8.10.11; 1.2.3.4.5.6.7.8.10.11; and 1.2.3.4.5.6.7.8.9.10.11. Comparing to the earlier data, the gene 9 appeared in the population.

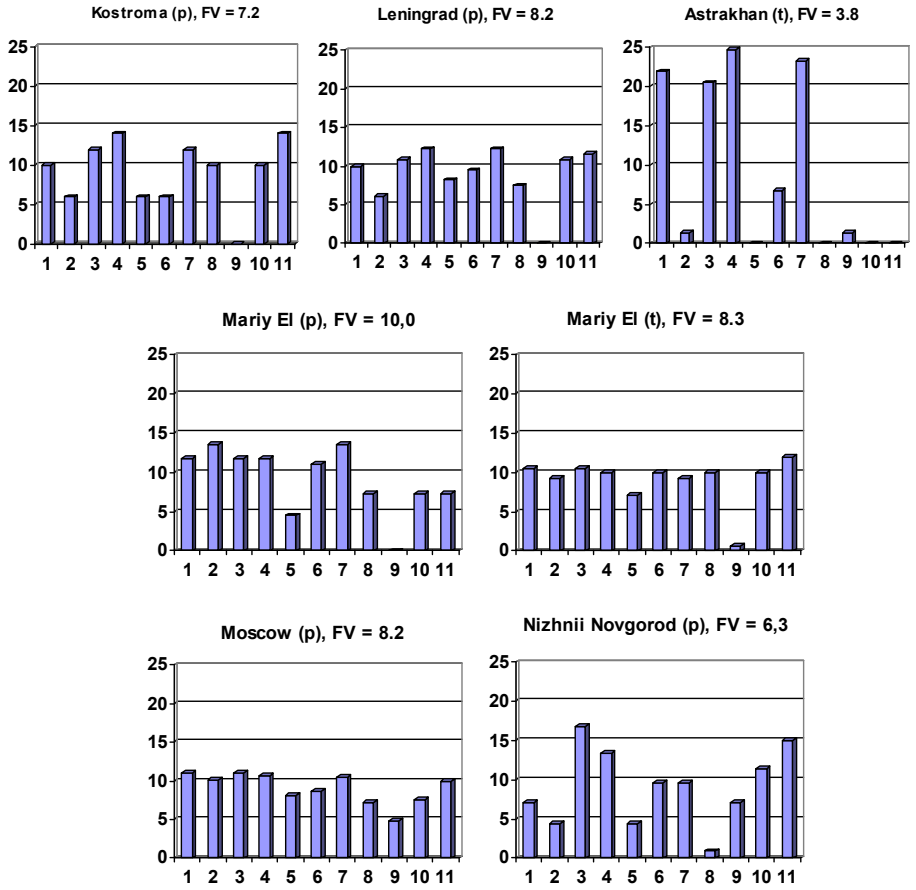

Fig. 5. *Frequencies of separate virulence genes in the studied P. infestans populations. FV, factor of virulence; (p) and (t) mean "potato" and "tomato" populations, respectively.*
The population from the Nizhnii Novgorod region consisted mainly of complex races, containing 6-11 virulence genes (57.9%). Like the Moscow population, this population also contained the gene 9; it is also interesting that the frequency of gene 8 was unusually low. The FV value (6.3) was lower than for the above-mentioned populations (7.2-10.0).
In the case of the Astrakhan population, we revealed a significant predominance of virulence genes 1, 3, 4, 7. Genes 5, 8, 10, and 11 were not revealed; the frequencies of genes 2 and 9 were very low. The FV value for this region was very low (3.8), and the most frequent race was the race 1.3.4.7 (47%). Thus, this population significantly differed from other ones.
In general, one should note that some of the tested populations contain the gene 9, which was not determined in the Russian P. infestans populations during our earlier studies.

Metalaxyl resistance

The results of the analysis of the studied *P. infestans* populations for their metalaxyl resistance are shown in Fig. 6.

Both Mariy El populations were represented by only susceptible isolates; the most of isolates from Kostroma and Moscow regions were also susceptible (98 and 58%, respectively). The percent of susceptible strains in the Moscow "potato" population significantly decreased comparing to the

earlier data [9] (from 78 to 58%). Astrakhan "tomato" and Leningrad populations were moderately resistant (100% and 76%, respectively). Finally, Nizhnii Novgorod population consisted mainly of the resistant isolates (73%).

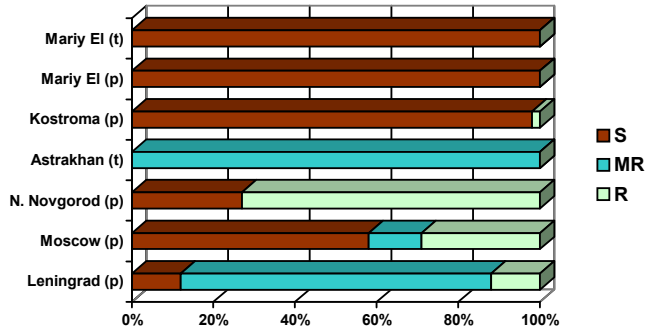

Fig. 6. *Frequency of P. infestans strains with different level of the metalaxyl resistance in different regions of European Russia. S, susceptible strains, MR, moderately resistant strains, R, resistant strains; (p) and (t) mean "potato" and "tomato" populations, respectively.*

CONCLUSIONS

The comparison of phenotypic and genotypic characteristics of *P. infestans* population of the Moscow region in 2008-2009 with the similar data, obtained in 1997-1998 showed that some significant changes occurred within a 10-year period. The frequency ratio of Ia and IIa MtDNA haplotypes shifted from 36:64 to 55:45, increasing the percent of Ia type. The A1:A2 ratio in both potato and tomato *P. infestans* populations of the region shifted from 72:28 (potato) and 88:12 (tomato) to 61:35.4 and 65:35, respectively, i.e. the percent of A2 strains increased. The virulence gene 9 appeared in the current population, which now contains all 11 virulence genes. The percent of susceptible strains in the "potato" population decreased from 78 to 58%.

The analysis of the studied populations showed that the Leningrad, Astrakhan, and Nizhni Novgorod populations are rather uniform. The Astrakhan "tomato" population has a unique pattern of virulence gene frequencies and seems to be monoclonal. The Moscow population has the highest diversity; a high diversity level was also observed for the Mariy El and Kostroma populations that can be explained by the fact these 3 regions represent potato-growing regions with a high volumes of imported seed potato.

The data obtained for the studied "potato" and "tomato" populations shows the first ones have a greater diversity in some parameters, such as the MtDNA haplotype and Pep1/Pep2 loci.

Most of the analyzed populations contained the virulence gene 9, which was not revealed in the populations, studied in our previous investigation.

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