PHYTOPHTHORA INFESTANS POPULATIONS FROM THE EUROPEAN PART OF RUSSIA: GENOTYPIC STRUCTURE AND METALAXYL RESISTANCE

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SUMMARY

Populations of the potato and tomato late blight pathogen from different regions of the European part of Russia were studied for their genotypic structure and metalaxyl resistance. The majority of the studied populations showed a high genotypic diversity, i.e. strains of A1 and A2 mating types, Ia and IIa mitochondrial DNA haplotypes, and different genotypes at Pep1 and Pep2 loci were identified. The highest genotypic diversity was registered among *P. infestans* populations from the North Caucasus, Mariy El Republic, and the Moscow region. The obtained data confirm a high probability of crossing and hybridization within the most part of the field populations of *P. infestans* from the European part of Russia. Most of the studied populations included strains with high and low metalaxyl resistance, the incidence of resistant strains depending on the use of metalaxyl-containing fungicides.

Key words: late blight, potato protection, oomycetes, population genetics, oospores

INTRODUCTION

Late blight is one of the most dangerous and economically significant potato and tomato diseases in many countries. During late blight epidemics, potato yield losses, caused by the early leaf decay and the rotting of infected stored tubers, can exceed 50%. In the case of favorable weather conditions and lack of chemical protection, the late blight pathogen is able to kill the tops of potato plants within 15-20 days (Fry, 2008). All macrocarpous tomato varieties, cultivated in Russia, are also subjected to the severe late blight infection with yield losses reaching 80-100%. Late blight control is complicated because of the high variability of the pathogen, *Phytophthora infestans* (Mont.) de Bary that causes the appearance of new strains, resistant to fungicides and highly aggressive towards the resistant potato and tomato cultivars.

At the end of the 20th century, a series of significant changes were registered in the structure of *P. infestans* populations. The first global changes took place in the 80s; they reflected a global trend, representing the replacement of the widespread US-1 clone with other genotypes (Fry *et al*., 1993). The last time when the strains with the US-1 genotype were observed in the Moscow region on tomato plants was in 1993 (Elansky *et al*., 2001). Since 1993, *P. infestans* populations including strains of both mating types with various genotypes, have prevailed in the European part of Russia (Elansky *et al*., 2001; Elansky and Smirnov, 2003; Amatkhanova *et al*., 2004; Shein *et al*., 2009).

Another wave of global changes in the *P. infestans* populations of the European part of Russia is still developing since the end of 1990s. These changes include an earlier start of the outbreaks, a general increase in the aggressiveness of *P. infestans* strains, and the free migration of isolates between potato and tomato plants (Elansky *et al*., 2007). Highly virulent strains have become widespread and reported from both tomato and potato crops (Amatkhanova *et al*., 2004; Shein *et al*., 2009). In some cases, infected tomato seedlings started epidemics on potato.

Current changes in *P. infestans* populations require a continuous adaptation of plant protection actions. Durable results in potato protection can be achieved only upon a fundamental knowledge of *P. infestans* biology, resistance to the chemical reagents, and virulence to the cultivated potato cultivars. Such knowledge is strongly dependent on the permanent monitoring of the population structure of plant pathogens.

The current study was undertaken to evaluate the genotypic structure of *P. infestans* populations from the distant regions of the European part of Russia and its relationship with the distribution of metalaxyl-resistant strains. Preliminary results were obtained for a set of independent markers (mating type, allozyme banding patterns at two peptidase loci (Pep1 and Pep2), and mitochondrial DNA (mtDNA) haplotype commonly used in population studies of the late blight pathogen in the years, when the majority of samples were collected. A microsatellite (SSR) study

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Fig. 1. Location of sampling sites in the European part of Russia (see Table 1).

of the studied *P. infestans* populations is now underway, whose results are planned to be published later.

MATERIALS AND METHODS

In this study we analyzed 567 *P. infestans* strains, isolated in 2000-2009 from infected samples, collected in the Vologda, Ryazan, Leningrad, Moscow, Astrakhan, Kostroma, Smolensk, and Tula regions, Mariy El, Mordovia, Northern Osetia, and Ingushetia republics, and the Stavropol Territory (Fig. 1; Table 1). Blighted samples were collected from plants in a distance of 10 or more meters from each other.

Fungicide resistance assessment. The fungicide resistance of collected samples was assessed on oat agar medium, supplemented with Ridomil (containing 25% metalaxyl), according to Shattock (1988). The final metalaxyl concentrations were 10 and 100 µg/ml. Inoculation of the tested fungal isolates on the fungicide-containing medium was carried out using agar blocks. All experiments were repeated three times. Using averaged values of the colony diameters, the ratio was calculated of the colony diameters on the fungicide-containing medium to the colony diameters on the medium without the fungicide:

$$K = D_{fung}/D_{contr}.$$

When the K value did not exceed 0.1 at a fungicide concentration equal to 10 µg/ml, the strain was considered as susceptible (S); if the K value was between 0.1 and 0.6, the strain was considered as semi-resistant (SR). When the K value exceeded 0.6 and the strain was able to grow on the fungicide-containing medium at a fungicide concentration equal to 100 µg/ml, the strain was considered as resistant (R).

Table 1. Sampling sites and dates of collection of *Phytophthora infestans* isolates from potato/tomato plants.

Sampling site	Abbreviation	Treatment with metalaxyl	Sampling date	Host plant*	Number of isolated *P. infestans* strains
Vologda region	0VP	untreated	July 2000	P	12
Ryazan region	0RP	untreated	August 2000	P	22
Moscow region	1MP	untreated	August 2001	P	26
	1MT	untreated	August 2001	T	16
Republic of Mordovia	1MST	untreated	August 2001	T	21
The Stavropol Territory	1SKP	treated	July 2001	P	25
Northern Osetia Republic	1SOP	no data	July 2001	P	25
Tula region	1TP	untreated	July 2001	P	33
Leningrad region	2LP	treated	August 2002	P	9
Republic of Ingushetia	2IP	no data	July 2002	P	15
Mariy El Republic	7MET	untreated	August 2007	T	89
	7MEP	untreated	August 2007	P	21
Leningrad region	8LP	untreated	July 2008	P	22
Kostroma region	8KP	untreated	August 2008	P	84
Moscow region	8MP	untreated	August 2008	P	45
Astrakhan region	8AT	treated	August 2008	T	33
Smolensk region	9SP	treated	August 2009	P	49
Kostroma region	9KP	untreated	July 2009	P	20

* P and T indicate potato and tomato, respectively.

Mating type determination. The mating type was determined by pairwise growing isolates on oat agar with the known reference strains of A1 and A2 mating types. Petri dishes were incubated in the dark at 18°C for 14 days, then the presence/absence of oospores at the points of hyphal contact was determined under the light microscopy. When the tested isolate generated oospores only with the A2 reference strain, it was referred to as A1 type. If the isolate generated oospores only with the A1 reference strain, it was referred to as A2 type. Isolates, generating oospores with both reference strains, were referred to as A1A2 type. Isolates that did not generate oospores with both reference strains, were referred as 00 type.

Allozyme analysis. To determine the allozyme banding pattern at two peptidase loci (Pep1 and Pep2) and glucose-6-phosphate isomerase locus (Gpi1), *P. infestans* isolates were cultivated on liquid pea medium in the dark for 8-14 days at 18ºC. When the amount of mycelium became sufficient, the allozyme spectrum was determined using Helena Labs cellulose acetate gels in accordance with the manufacturer's recommendations (Hebert and Beaton, 1993). Gel profiles of Pep1 and Pep2 loci are shown in Fig. 2.

DNA isolation. Mycelium grown on liquid pea medium, was ground in liquid nitrogen and lysed in CTAB buffer. DNA was purified using chloroform and stored in deionized water at –20ºC.

Mitochondrial DNA haplotyping. MtDNA haplotypes were identified using the method of Griffith and Shaw (1998), which allows for the detection of four mtDNA haplotypes (Ia, Ib, IIa, and IIb). The primers used for amplification of the P2 region were F2 (5'-TTCCCTTT-GTCCTCTACCGAT-3') and R2 (5'-TTACGGCGGTT-TAGCACATACA-3'), whereas primers F4 (5'-TGGT-CATCCAGAGGTTTATGTT-3') and R4 (5'-CCGATAC-CGATACCAGCACCAA-3') were used to amplify the P4 region. The PCR cycling programme used was the same for both primer combinations: 1× (90°C for 9 min), 40× (90°C for 30 sec, 52°C for 30 sec, 72°C for 90 sec), 1× (72°C for 5 min). For restriction enzyme analysis 10 µl of the PCR reaction mix was mixed with 6 µl of water, 2 µl of the appropriate 10× Buffer, and 2 µl (10 units/µl) of enzyme (*Msp*I for region P2 and *Eco*RI for P4). Digestions were performed at 37°C overnight. Ten microlitres of the digested DNA sample were loaded into a 2% agarose gel in 1× TBE buffer, containing 0.1 µl/ml ethidium bromide. The gel was run at 10 V/cm and restriction patterns were visualized using an UV transilluminator.

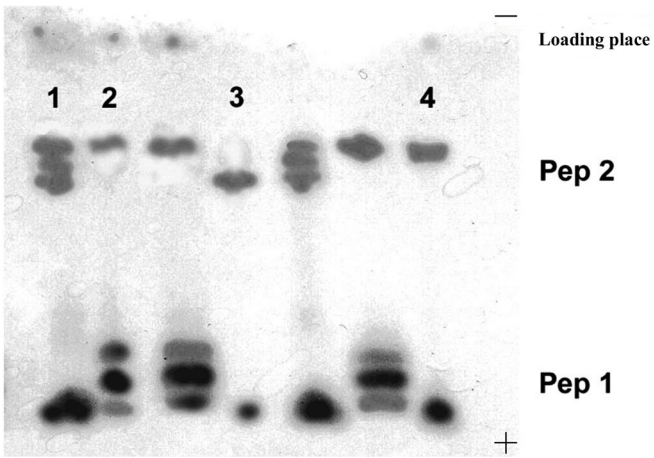

Fig. 2. Electrophoregrams of the peptidase loci Pep1 and Pep2.

RESULTS

Markers variability. *Mating type.* Strains of both mating types were observed in most of the *P. infestans* populations studied, however their ratio varied for different regions. Populations from Tula (1TP), Leningrad (8LP), and Astrakhan (8AT) comprised only strains of A1 mating type (Table 2). Populations with strains of the A2 mating type only were not found. Strains, able to generate oospores with both reference strains (A1A2 type) were present in the populations from the Moscow (8MP) and Kostroma (8KP) regions, the Stavropol Territory (1SKP), and Republic of Ingushetia (2IP). Populations from the Mariy El republic (7MET) and the Stavropol Territory (1SKP) had strains which did not generate oospores with both reference strains (00 type).

Pep1 and Pep2 loci. In the studied strains, the Pep1 locus was present in two genotypes 100/100 and 92/100 (Fig. 2, lines 1 and 2, respectively), with a prevalence of the first one. The more rare 92/100 genotype was identified in strains from the Moscow and Kostroma regions, Northern Osetia, the Stavropol Territory (1SKP), Republic of Ingushetia (2IP), and Mariy El Republic (7MEP). The 92/92 genotype was not found. The Pep2 locus occurred in three genotypes: 100/100, 100/112, and 112/112 (Fig. 2, lines 1 and 3, respectively). Populations from the Stavropol territory (1SKP), Northern Osetia (1SOP), Republic of Ingushetia (2IP), Mariy El Republic (7MET), Moscow region (8MP), and Belarus (BP) included all three genotypes. Interestingly no 100/112 heterozygotes were detected in the Kostroma region (8KP), though the population from this region comprised strains with the 100/100 genotype and A2 mating type and strains with the 112/112 genotype and A1 mating type (Table 2). This fact might indicate no sexual process and hybridization took place in the studied potato fields. A very low percent of strains with the 100/112 genotype was found in Mariy El Republic (7MET), though they were represented by both mating types and by both 100/100 and 112/112 genotypes.

The Gpi1 locus of glucose-6-phosphate isomerase, traditionally used in population studies, was analyzed in 129 isolates, collected in 1997-1998 in European, Siberian and

Table 2. *Phytophthora infestans* strains with different genotypes in the tested populations.

№	Genotype*	Number of *P. infestans* strains with the certain genotype in the population:																	Number of populations containing the genotype
		0VP	0RP	1MP	1MT	1MST	1SKP	1SOP	1TP	2LP	2IP	7MEP	7MET	8LP	8MP	8KP	8AT	9SP	
1	A2, 100/100-100/100, IIa	3	1	2	4		5	3		2		6				4			9
2	A1, 100/100-100/100, Ia	1		2			3	7				1	2		5		28	3	9
3	A1, 100/100-100/100, IIa	2	10				3	2		1	6				13				7
4	A2, 100/100-100/112, Ia			4	6		1						1		2			31	6
5	A1, 100/100-100/112, IIa	2								3		1	2		1				5
6	A2, 100/100-100/112, IIa						1	6			3	5	1						5
7	A2, 100/100-100/100, Ia					10						1	16		8				4
9	A1, 100/100-112/112, Ia												12	12	1	41			4
8	A1, 100/100-100/112, Ia					3			10		1								3
10	A2, 100/100-112/112, IIa						1	1					1						3
11	A2, 92/100-100/100, Ia										1					2			2
12	A2, 92/100-100/100, IIa						1					1							2
13	A1, 100/100-112/112, IIa		2					3											2
16	A1, 92/100-100/100, Ia														3				1
17	A1A2, 100/100-100/112, IIa														1				1
18	A1, 92/100-100/100, IIa							1											1
19	A2, 100/100-100/112, IIa												1						1
20	A2, 100/100-112/112, Ia												1						1
21	A1A2, 92/100-100/100, IIa						1												1
22	00, 100/100-100/100, IIa						1												1
23	00, 100/100-112/112, Ia												1						1
24	A2, 92/100-100/112, IIa						1												1
25	A1A2, 100/100-100/100, IIa						1												1
26	A1A2, 100/100-100/100, Ia						1												1
27	A1A2, 100/100-100/112, IIa						1												1
Number of tested strains in the population		8	13	8	10	13	21	23	10	6	11	15	38	12	34	47	28	34	
Number of genotypes in the population		4	3	3	2	2	13	7	1	3	4	6	10	1	8	3	1	2	

* data shown as follow: mating type, Pep1-Pep2 genotypes, mtDNA haplotype.

Far Eastern parts of Russia (for location of sampling sites refer to Elansky *et al*., 2001), in 38 isolates collected in the European part of Russia in 2000-2001, and in 20 isolates from Belarus, collected in 2006-2007. All these isolates had the same 100/100 genotype, so no further analysis of the locus was carried out.

MtDNA haplotypes. The most part of the studied populations included strains of both haplotypes (Table 2). Populations from the Ryazan (0RP) and Leningrad (2LP) regions had only strains of the IIa type, whereas the populations from the Republic of Mordovia (1MST) and Tula (1TP), Leningrad (8LP), and Astrakhan (8AT) regions included strains of Ia type only. In the Mariy El Republic, *P. infestans* strains collected from potato fields comprised mainly the IIa haplotype, whereas strains collected from the tomato fields, included mainly the Ia haplotype. Haplotypes, Ib (reported previously by Elansky *et al*., 2001 in samples analyzed in 1993) and IIb were not observed (or recorded) during the present investigation.

Analysis of the independence of the studied markers. The chi-square analysis of the interference of the studied markers (mating type, Pep1 and Pep2 loci, and mtDNA haplotype) did not reveal any correlation between them ($p > 0.05$), which allowed conducting a genotypic analysis of the studied strains.

Genotypic structure. The genotypic structure analysis of the studied populations showed a high level of diversity in the populations from the Stavropol territory (1SKP), Northern Osetia (1SOP), Mariy El Republic (7MET and 7MEP), Moscow region (8MP), which included 6 or more different genotypes (Table 2). Populations from the Tula (1TP), Leningrad (8LP), and Astrakhan (8AT) regions were genetically uniform and probably monoclonal. Other populations comprised 2-4 different genotypes and were suggested as polyclonal. The most frequent genotypes were A2, 100/100-100/100, IIa and A1, 100/100-100/100, Ia (9 of 17 studied populations), A1, 100/100-100/100, IIa (7 populations), and A2, 100/100-100/112, Ia (6 populations).

Metalaxyl resistance. The analysis of metalaxyl resistance showed that susceptible strains prevailed in the most of the studied populations (Table 3). A high percentage of resistant strains was registered in the populations from the

Stavropol Territory (1SKP), Republic of Ingushetia (2IP), Moscow region (8MP), and Smolensk region (9SP). Single resistant isolates were also observed in the populations from the Northern Osetia (1SOP), Astrakhan (8AT) and Kostroma (8KP) regions. Moderately resistant strains were found in all studied regions, excluding Vologda (0VP), Ryazan (0RP), Kostroma (9KP), and Tula (1TP) regions, the Republic of Mordovia (1MST), and Mariy El (7MEP).

Table 3. Percentage of *Phytophthora infestans* strains with different levels of metalaxyl resistance.

Population	Percentage of *P. infestans* strains with a certain resistance level		
	S*	SR	R
0VP	100	0	0
0RP	100	0	0
1MST	100	0	0
1SKP	38	46	16
1SOP	90	5	5
1TP	100	0	0
2IP	53	27	20
7MEP	100	0	0
7MET	83	17	0
8MP	19	44	37
8LP	31	69	0
8AT	20	76	4
8KP	53	45	2
9SP	6	14	80
9KP	100	0	0
BP	79	13	8

* S: sensitive, SR: semiresistant, R: resistant.

DISCUSSION

The majority of the studied populations included strains of both mating types, different genotypes at Pep1 and Pep2 loci, two mtDNA haplotypes (Ia and IIa), and showed a high genotypic diversity, especially in the case of the populations from North Caucasus (1SKP and 1SOP), Mariy El Republic (7MEP and 7MET), and Moscow region (8MP). In the latter case, the high genotypic diversity found with the present investigation agrees with the results of the SSR and RG57 study of a subset of this population (Statsyuk *et al*., 2014).

A high genotypic diversity in *P. infestans* populations can be caused by different reasons, such as hybridization during the sexual process, intensive exchange of low quality infected seed materials, and the dispersal of sporangia in the atmosphere. The current population structure of *P. infestans* in Russia is suggested to be dependent on all these factors.

Analyzing the situation in Russia, one should take into account that more than 85% of the total potato yield is produced on small private farms and kitchen gardens, whose owners usually plant their own low-quality seed material, often consisting of unknown cultivars or a mixture of different cultivars. Sometimes farm owners buy tubers of table potato in superstores and use them as seed material; these tubers may have come from other regions of Russia or from abroad. As a rule, farm owners do not apply any protective treatments or perform incorrect treatments, using pesticides of unknown origin. When weather conditions are suitable, all these factors provide favorable conditions for late blight outbreaks.

When *P. infestans* strains with different mating types are present in the same plot, crossing, hybridization, and oospore formation may take place, which induce the development of new genotypes. During 1997-2008, we studied the mating type distribution in 41 *P. infestans* populations from small private farms and kitchen gardens, located at different sites of the Moscow region. The obtained data showed that most of the populations included both mating types. Only 12% of the populations comprised strains of only one mating type (S.N. Elansky, unpublished information).

To evaluate the crossing under natural conditions, a screening of oospores in the infected tissues of potato and tomato plants was carried out. Oospores were observed in potato and tomato leaves from many sites of the Moscow region, North Caucasus, and Mariy El republic. The oospore frequency was higher in the leaves with several late blight lesions, which were collected from the places where both mating types were observed, than in the leaves with only one lesion (Smirnov and Elansky, 1999; Amatkhanova *et al*., 2004; Shein *et al*., 2009). It was shown that the oospores generated from crossing of strains of different mating types, would overwinter in the soil and serve as a source of infection in the next season (Kuznetsova *et al*., 2010; Lehtinen *et al*., 2002; Bødker *et al*., 2006; Hermansen *et al*., 2002).

A high genetic and genotypic diversity of some populations indirectly confirmed the presence of the sexual process and hybridization in these populations. For example, North Caucasus populations (1SKP, 1SOP, 2IP) contained all three possible alleles of the Pep2 locus (two homozygotes and one heterozygote). The ratio of their frequencies corresponded to the Hardy-Weinberg equation at the significance level equal to 95% (Amatkhanova *et al*., 2004). Thus, outbreeding probably contributes to some extent to determine the ratio of genotype frequencies.

The results of our studies confirmed the occurrence of oospore formation and a high probability of crossing and hybridization in most of the *P. infestans* populations from the European part of Russia. Oospores, able to overwinter and survive under unfavorable conditions, could significantly account for the changes observed in tomato and potato populations of *P. infestans*. The aggressiveness of strains and their fungicide resistance decreased during the overwintering in tubers and the following latent period between tuber planting and appearance of the first late blight symptoms on potato leaves (Derevyagina *et al*., 1991). *P. infestans* isolates from potato were represented mainly by strains of the T0 race, which did not have a

Table 4. Percentage of *Phytophthora infestans* strains with and without the resistance gene (T0 and T1 races, respectively) among the strains, isolated from potato and tomato leaves.

Population	Host plant[a]	Number of tested *P. infestans* strains	% T0	% T1
Moscow region				
1999	T	16	44	56
	P	24	87	13
2003	T	65	66	34
	P	57	86	14
Other regions				
2001, Tula region (1TP)	P	18	6	94
2002, Republic of Ingushetia (2IP)	P	13	46	54
2002, Leningrad region (2LP)	P	10	30	70

[a] P and T = potato and tomato, respectively.

gene of virulence towards the cherry-like tomato cultivars (Table 4). However, the strains with the highest level of aggressiveness to tomato, had such virulence gene T1 (T1 race).

According to our data, such strains might be also very aggressive to potato. For example, the most aggressive population from the Tula region (1TP) was represented mainly by the T1 race (Table 4). At the same time, experiments with the infection of potato tubers with T1 strains and the mix of T0 and T1 strains showed that the survival of T1 strains in potato tubers is rather low; the T0 race predominates in seed tubers in spring (Dyakov *et al*., 1975; Rybakova, 1987). Since transition of strains from T0 to T1 and vice versa during the infection process was not observed (Lavrova *et al*., 2003), if the strains overwinter in the tubers, then they would have a low virulence towards tomatoes.

Until the 1990s, late blight outbreaks on tomato fields of the European part of Russia occurred significantly later than the appearance of the first symptoms on potato plants. Overwintering in the form of oospores is possibly the reason for the recent changes, such as the earlier start of epidemics, development of infection foci on tomatoes before their appearance on potatoes, general increase in the aggressiveness of tomato and potato strains, predominance of races with a higher number of virulence genes toward potato among the tomato strains, and the ease of the reciprocal infection between tomato and potato. Aggressive strains, belonging to the T1 race, were observed on potato in the beginning of the epidemic (1TP, 2LP, and 2IP populations; A.V. Filippov and V.G. Ivanyuk, personal communications). In some cases infected tomato seedlings caused a start of late blight epidemics on potato (A.V. Filippov, personal communication).

The presence of strains with different metalaxyl resistance levels in a population first of all depends on the use of metalaxyl-containing fungicides at any specific site or field. Metalaxyl resistance can easily appear even in the clonal lines (Elansky *et al*., 2001, 2007) and remains for a long time, since it does not influence significantly the competitive abilities of the strains (Gisi and Cohen, 1996).

Until 2008 metalaxyl-containing preparations were not sold in Russia in small packages. Therefore, they were not used on small private farms and kitchen gardens, from which more than 85% of the total potato production originates. *P. infestans* populations present in such farms were represented mainly by susceptible strains which exceeded 70%. During the whole studied period (2000-2009) we did not observe any population composed by resistant strains only on the private farms, whereas such populations were found in larger commercial fields. The same situation, with a higher frequency of susceptible strains on the private farms was observed in Great Britain (Day *et al*., 2004).

In the case of large commercial fields, the situation is quite different. There are all possible variants of the ratio of resistant, susceptible, and low-resistant strains, ranging from populations, represented only by highly resistant strains, to populations having only susceptible strains. The percentage of resistant strains depends only on the use of metalaxyl-containing preparations for plant protection.

The use of metalaxyl-containing preparations for growing of seed potato is prohibited in Russia. In fact, the data from the Table 3 show that the strains, collected from seed-growing potato fields (1TP, 8LP, 8KP, and 9KP), are mainly susceptible and low-resistant; the number of resistant strains is rather low. In the case of commercial fields, intended for growing of table potato, there can be different situations: resistant strains can either predominate (1SKP, 9SP), or be in a small number or even absent (0VP). The highest frequency of resistant strains was observed in the *P. infestans* population from the Smolensk region (9SP). Among 49 tested isolates, 39 (80%) were resistant. Potato tubers, planted in 9SP, were grown on the seed field 8KP with a very low percent of resistant strains. Tubers from that field were also planted in seed field in Kostroma region (9KP). In the case of the 9SP field, the owner applied Ridomil Gold MZ twice; the treatments were performed between intensive rains, which, possibly, washed the chemical away. In the seed potato field 9KP in which only Acrobat MZ was applied, no metalaxyl resistant strains were found (Table 3).

The appearance of metalaxyl-resistant *P. infestans* strains in the Smolensk region (9SP) could result from either an increase in the resistance of initially susceptible strains present in seed material, or the introduction of resistant strains from local potato fields. The first option seems to be rather improbable, because the genotyping of the 9SP and 8KP populations collected from the same seed material grown in the Smolensk and Kostroma regions, respectively, showed some differences between these populations. The absence of coinciding genotypes in these two regions (Table 2) and a significant difference in the percentage of resistant isolates (Table 3) makes it possible to suppose that resistant strains collected in the

Smolensk region probably originated from the surrounding fields, but not from the initial seed material, thus they had a local origin.

The presence of strains of both A1 and A2 mating types and detection of oospores in the samples from some *P. infestans* populations confirms the existence of a sexual process and hybridization in these populations. The hybridization results in the generation of new genotypes; it occurs more intensively on small untreated private farms. The further expansion of seed material and sporangia (via the air) promotes an increase in the diversity of *P. infestans* populations on the surrounding farms and fields, including large commercial fields of seed and table potatoes.

In the case of intensive treatment of fields belonging to agricultural companies with metalaxyl-containing preparations, an accumulation of resistant *P. infestans* strains takes place. Spreading with the wind and with seed potato, these strains appear on private farms, where participate in the sexual process and serve as the donors of resistance genes. A mass generation of new genotypes and an absence of any treatments with metalaxyl-containing fungicides prevent an accumulation of metalaxyl-resistant strains on private farms. Therefore, *P. infestans* populations, existing on such small properties can be considered not only as the global source of a high diversity of *P. infestans* strains, but also as a kind of buffer, which reduces the fraction of highly resistant strains. Now in Russia we observe a tendency to reduce the area of potato plots in the sector of small private farms. This trend can result in an increase in the percentage of *P. infestans* strains resistant to metalaxyl and other fungicides, in Russian tomato and potato fields.

The present data confirm the high probability that crossing and hybridization take place in *P. infestans* populations of the European part of Russia. Hybridization results in the generation of new genotypes, which can be more aggressive for plants than the parental strains. Thus, it is important to continue to monitor the genotypic changes in *P. infestans* populations; only long-time observations make it possible to predict the development of the disease and elaborate the optimal measures for disease management.

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