Fungicide Resistance of Russian *Phytophthora infestans* strains

MARINA A. POBEDINSKAYA[1], SERGEY N. ELANSKY[1], NATALIA V. STATSYUK[2] & MIKHAIL P. PLYAKHNEVICH[3]

[1]Lomonosov Moscow State University, Moscow, 119899 Russia; e-mail: elansky@yahoo.com
[2]All-Russian Research Institute of Phytopathology, Bolshie Vyazemy, Moscow region, 143050 Russia
[3]Scientific and Practical Center for Potato, Vegetable, and Fruit Growing, Samokhvalovichy, Minsk region, 223013 Belarus

SUMMARY
P. infestans strains have been isolated in pure culture from blighted samples, collected in the Moscow, Leningrad, Astrakhan, Smolensk, and Kostroma regions, Mariy El Republic, and Belarus. The fungicide resistance of collected strains has been tested in Petri dishes with agar medium. The most efficient fungicides are azoxystrobin and dimethomorph. The efficiency of fluazinam, chlorothalonil, and mancozeb is also rather high. Therefore, the application of these fungicides in recommended dosages can provide a successful potato late blight control. The strains, highly resistant to metalaxyl, has been revealed in the Moscow and Smolensk regions, and also in Belarus.

KEYWORDS
Phytophthora infestans, late blight, fungicides

INTRODUCTION
Late blight is a dangerous disease of potato and tomato, which is typical for many countries and causing significant yield losses. The late blight control is complicated by extremely high variability of its agent, *Phytophthora infestans* (Mont.) de Bary, that results in the appearance of fungicide-resistant strains, highly aggressive to the earlier resistant potato cultivars.

The main way to control late blight is the chemical protection of crops, i.e. fungicidal treatments of fields. However, the efficient action of fungicides is possible only in the case if pathogen populations do not have or have a very low number of highly resistant strains. The assessment of the resistance of the pathogen to the used fungicides is carried out not too often, and such data are absent for many regions of Russia and Belarus.

In our study we assessed the resistance of late blight strains from Belarus and some distinct regions of the European part of Russia to several popular fungicides, such as metalaxyl (systemic fungicide), azoxystrobin (limited systemic fungicide), dimethomorph (translaminar fungicide), fluazinam, chlorothalonil, and mancozeb (contact fungicides).

PPO-Special Report no. 15 (2012), 243- 248

MATERIALS AND METHODS

Collection of isolates

In our study we used *P. infestans* isolates, collected in 2007-2009 from tomato and potato fields in the Leningrad, Moscow, Astrakhan, Kostroma, and Smolensk regions, Mariy El Republic, and Belarus (Fig. 1, Table 1). One isolate was isolated from each leaf lesion.

Figure 1. Sampling site locations on the European part of Russia. 1, Leningrad region; 2, Moscow region; 3, Kostroma region; 4, Mariy El Republic; 5, Astrakhan region; 6, Belarus.

Posters

Table 1. Location and time of the sampling of blighted plants and the number of strains, isolated into the pure culture

Sampling location	Sampling date	Fungicidal treatment	Host plant	Number of isolated strains
Leningrad region, Belogorka village, fields of the Leningrad Research Institute of Agriculture	07.2008	–	PL*	22
Kostroma region, Minskoe village, fields of the Kostroma Research Institute of Agriculture	08.2008 08.2009	+	PL	84
Mariy El Republuc Yoshkar-Ola outskirts, private gardens	08.2007	–	TF**	89
	08.2007	–	PL	21
Moscow region, Lyubertsy district, fields of the All-Russian Potato Research Institute	08.2008	–	PL	45
Smolensk region, Safonovo district, Izdeshkovo village	08.2009	+	PL	49
Astrakhan region, Kamyzyak district	08.2008	+	TF	33
Belarus, different regions	2006-2007	+	PL***	78

*PL, potato leaves.
**TF, tomato fruits.

Isolation of strains into the pure culture

Isolation of strains into the pure culture was carried out using wet chambers. After the appearance of the fruiting on the surface of an infected sample, it was microscoped, and zoosporangia were collected using a microbiological needle with a piece of agar medium on its tip; during the collection of zoosporangia, the agar block did not touch the sample surface. Zoosporangia were put onto oat agar medium, supplemented with penicillin (1000 μg/ml) and incubated until the diameter of a colony reached 4-5 cm. Then a piece of mycelium from the edge of the colony was transferred into another Petri dish with the same medium.

Fungicide resistance assessment

The resistance of isolates to fungicides was assessed on oat agar medium, supplemented with the corresponding fungicide at various concentrations (0.1, 1, 10, 100, and 1000 μg/ml), and on the medium without any fungicide (control). The experiments were made in three repetitions. For each isolate we determined the EC50 value, i.e. the concentration of a fungicide, causing a twofold delay in the colony growth rate.

RESULTS AND DISCUSSION

Fungicides significantly differ concerning the chance of appearance of fungicide-resistant strains in a pathogen population. An increase in the resistance to multi- and oligo-site fungicides occurs rather slowly and involves a step mutagenesis. Probably, due to this fact, the treatment of plants with mancozeb, fluazinam, chlorothalonil, and dimethomorph did not result in the appearance of highly resistant strains in a population. The analysis of a mancozeb resistance did not revealed any strains, which EC_{50} level would exceed 31 μg/ml, though several populations included strains, which characteristics were close to this level (Table 2). Probably, this level represents a threshold value, and strains with a higher resistance level are nonviable or noncompetitive in agrocenoses.

The resistance of *P. infestans* strains to azoxystrobin should be discussed separately. In all studied populations we observed only highly susceptible isolates, though, according to other authors, the risk of development of the azoxystrobin resistance is considered to be rather high. Probably this fact is connected with a low level of application of the Quadris preparation in the European part of Russia, since this preparation is registered only for the treatment of tomato. We specially examined strains, isolated from commercial tomato fields in the Astrakhan region, where azoxystrobin and krezoxym-methyl, another strobilurine fungicide, are widely used. However, in this case we also observed only highly susceptible isolates. On the other hand, in the case of potato fields in the European part of

Russia, farmers often use famoxadon and phenamidon; resistance to these preparations is crossed with that to azoxystrobin (Bartlett *et al.*, 2002). It seems that the development of the azoxystrobin resistance in *P. infestans* strains is a very rare event.

The analysis of a metalaxyl resistance showed that the most of the studied populations were represented by susceptible strains. The EC50 levels of isolates, collected from the Astrakhan and Leningrad regions and Mariy El Republic, did not exceed 5 µg/ml. In the case of the Kostroma region, we did not observe any isolates, which EC_{50} value would exceed 40 µg/ml, though in 2009 we specially collected samples from fields, treated with Ridomil Gold MC. Among 64 isolates, collected in different regions of Belarus from commercial potato fields, which are usually treated with phenylamide-containing fungicides, only five had the EC50 level, exceeding 10 µg/ml; only two of them were highly resistant (EC50 > 100 µg/ml). In the case of the Moscow region, samples were collected from an untreated field, surrounded by commercial potato fields. Among 31 examined isolate, 8 had EC50 > 100 µg/ml. The highest percentage of highly resistant isolates was observed in the Smolensk population. Among 49 tested isolates, 28 were highly resistant (EC50 > 100 µg/ml); only 5 isolates were susceptible (EC50 < 10 µg/ml).

The appearance of metalaxyl-resistant strains in the Smolensk region probably was caused by either an increase in the resistance of initially susceptible strains, or the introduction of resistant strains from other potato fields. The second version seems to be rather unlikely, since we did not reveal any highly resistant isolates in the Kostroma region (the source of a seed material for the Smolensk region), even on the fields, treated with metalaxyl-containing preparations. In addition, the study of the allozyme structure of peptidase showed some differences between resistant strains from the Smolensk region and the strains, collected in 2008 in the Kostroma region. Therefore, resistant Smolensk strains have rather local origin.

A high genotypic diversity of *P. infestans* populations, observed in some regions of Russia (Amatkhanova *et al.*, 2004; Shein *et al.*, 2009; Elansky *et al.*, 2001, 2003), can provide the selection of highly resistant and aggressive strains in the case of intensive and wrong fungicidal treatments; the appearance of such strains is able to stultify the efficiency of a crop protection. First of all, it concerns the application of metalaxyl-containing preparations, since even if only 3% of resistant forms present in a population, it will be enough for the rapid increase in the number of resistant strains in the case of an uncontrolled metalaxyl application.

In general, according to the obtained results, the most of strains, composing Russian *P. infestans* populations, are susceptible to the most popular fungicides and, therefore, the resistance of even the most resistant isolates can be successfully overcome by the use of fungicides in the dosages, recommended for Russia and Belarus. The only exception is metalaxyl resistant isolates, which control with phenylamide-containing fungicides can be inefficient in the Moscow and Smolensk regions and also Belarus.

Table 2. Fungicide resistance of P. infestans strains from different regions of Russia and Belarus

Fungicide	Sampling site	Number of tested isolates	EC_{50} variability, µg/ml	Average EC_{50} level, µg/ml	Nuimber of strains with different EC_{50} levels			
					<1	1–10	10–100	>100
Mancozeb	Moscow region	23	0,6 – 22,4	5,51	5	16	2	0
	Leningrad region	19	0,98 - 26	10,9	1	10	8	0
	Smolensk region	—*	—	—	—	—	—	—
	Kostroma region	25	0,5 – 25,6	6,48	3	20	2	0
	Astrakhan region	20	0,6 – 18,6	5,31	4	14	2	0
	Mariy El Republic	45	0,5 – 27	8,42	2	30	13	0
	Belarus	57	0,64 – 30,8	10,2	6	31	20	0
Methalaxyl	Moscow region	31	0,52 - 398	100,0	14	4	5	8
	Leningrad region	12	0,54 – 4,7	2,0	7	5	0	0
	Smolensk region	48	0,51 – 380,0	168,9	2	2	12	32
	Kostroma region	52	0,51 – 38,75	1,67	44	7	1	0
	Astrakhan region	25	0,51 – 2,85	0,85	22	3	0	0
	Mariy El Republic	47	0,5 – 4	0,76	42	5	0	0
	Belarus	64	0,5 – 151,5	5,5	55	4	2	2
Azoxystrobin	Moscow region	15	0,05 – 0,07	0,05	15	0	0	0
	Leningrad region	10	< 0,05	< 0,05	10	0	0	0
	Smolensk region	10	< 0,05	< 0,05	10	0	0	0
	Kostroma region	14	0,05 – 0,07	0,05	14	0	0	0
	Astrakhan region	10	< 0,05	< 0,05	10	0	0	0
	Mariy El Republic	10	< 0,05	< 0,05	10	0	0	0
	Belarus	27	< 0,05	< 0,05	27	0	0	0
Chlorothalonil	Moscow region	8	0,51 – 0,79	0,6	8	0	0	0
	Leningrad region	5	0,53 – 5,5	3,0	2	3	0	0
	Smolensk region	8	0,64 – 4,95	2,17	4	4	0	0
	Kostroma region	6	0,81 – 11,5	4,48	3	2	1	0
	Astrakhan region	10	0,55 – 5,5	3,18	3	7	0	0
	Mariy El Republic	4	1,82 – 3,45	2,67	0	4	0	0
	Belarus	23	0,69 – 16,75	4,85	3	19	1	0
Fluazinam	Moscow region	8	0,53 – 5,24	2,03	4	4	0	0
	Leningrad region	5	0,59 – 5,34	2,36	2	3	0	0
	Smolensk region	7	0,52 – 6,83	2,48	4	3	0	0
	Kostroma region	8	0,53 – 5,99	1,7	6	2	0	0
	Astrakhan region	9	0,75 – 4,26	1,92	5	4	0	0
	Mariy El Republic	4	4,21 – 8,43	5,51	0	4	0	0
	Belarus	21	0,5 – 30,12	6,98	3	14	4	0
Dimethomorph	Moscow region	9	0,05 – 0,09	0,06	9	0	0	0
	Leningrad region	5	0,05 – 0,07	0,06	5	0	0	0
	Smolensk region	7	< 0,05	< 0,05	7	0	0	0
	Kostroma region	7	0,05 – 0,06	0,05	7	0	0	0
	Astrakhan region	7	0,05 – 0,09	0,06	7	0	0	0
	Mariy El Republic	5	0,05 – 0,06	0,06	5	0	0	0
	Belarus	34	< 0,05	< 0,05	34	0	0	0

*Not studied.

REFERENCES

F. Amatkhanova, Yu. Dyakov, Ya. Petrunina, M. Pobedinskaya, S. Elansky, I. Kozlovskaya, B. Kozlovsky, E. Morozova and A. Smirnov, 2004. Characteristics of *Phytophthora infestans* populations of the Northern Caucasus. Mycology and Phytopathology 38: 71-78 [in Russian].

S. Shein, D. Milyutina, I. Kozlovskaya, E. Morozova, M. Pobedinskaya and S. Elansky, 2009. Genotypic variability of *Phytophthora infestans* in the Mariy El Republic. Mycology and Phytopathology 43: 358-363 [in Russian].

D. Bartlett, J. Clough, J. Godwin, A. Hall, M. Hamer and B. Parr-Dobrzanski, 2002. The strobilurin fungicides. Pest Manag. Sci. 58: 649-642.

S. Elansky and A. Smirnov, 2003. Second locus of Peptidase as a marker for genetic investigations of *Phytophthora infestans*. Botanica Lithuanica, 9: 275-283.

S. Elansky, A. Smirnov, Yu. Dyakov, A. Dolgova, A. Filippov, B. Kozlovsky, I. Kozlovskaya, P Russo, C. Smart and W. Fry, 2001. Genotypic analysis of Russian *Phytophthora infestans* isolates from the Moscow region, Siberia and Far East. J. Phytopathol 149: 605-611.