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Resistance of Russian Strains of *Phytophthora infestans* to Fungicides Metalaxyl and Dimethomorph

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Abstract—In total, 2000 *P. infestans* isolates collected during 1988–2004 in different regions of Russia were tested for resistance to metalaxyl. In the majority of field populations, the frequency of resistant strains decreased after 1993–1994. This might be related to changes in the potato industry in Russia. Potato production was concentrated in small private gardens. The part of resistant strains in populations from small private patches was less than that in large commercial fields. Small private gardens became a great source of sensitive genotypes. In recent years, the part of resistant strains in the majority of field populations was less than 30%. A small number of resistant strains in a population occurs even if there has been no treatment with metalaxyl-containing preparations for a long time. In some populations, the frequency of resistant strains has increased, depending on treatments. Variation in the level of resistance to metalaxyl in one clonal lineage is shown. Resistant strains occurred in potato leaves and tubers, and in tomato leaves. They were rare in tomato fruits. Probably, the sensitive strains affecting fruits have a selective advantage. More than 370 strains from different regions were tested for resistance to dimethomorph-containing preparations. Resistant strains were not detected.

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Metalxyl is a systemic acylalanine fungicide belonging to phenylamides devised by specialists of *Ciba–Geigi* Co against Peronosporales. The effect of metalaxyl is based on blocking of RNA polymerase 1 suppressing polymerization of ribosome RNA due to impossibility of uridine nucleotide being inserted into the molecule chain (Davidse et al., 1991). It is shown that resistance to metalaxyl is controlled by one codominant nuclear locus. For resistance to metalaxyl to appear, one or several mutations are sufficient (Gisi and Cohen, 1996). The frequency of mutations is 6.9×10^{-6}, which is much higher than the frequency of mutations of resistance to the widely known antibiotics oxytetracycline (6.9×10^{-8}) or blasticidin S (7.2×10^{-8}). Mutations of resistance to metalaxyl greatly increase the resistance level of a strain and do not interfere with its ability to compete in the agrocenosis.

Resistance to fungicide compounds based on phenyamides (active substances metalaxyl, mephenoxam, and oxadixyl, which are included in various mixed preparations such as ridomyl, sandophan, ridomyl–gold, etc.), was for the first time recorded in a population of the causative agent of phytophthorosis in the Netherlands and Israel (Davidse et al., 1981, 1983; Cohen and Reuveni, 1983) in the early 1980s. Since then, many countries perform permanent monitoring of strains resistant to phenylamide preparations in populations of *Phytophthora infestans.* In addition to evaluating the prospects of applying phenylamide-containing preparations, resistance to them has become a widely used marker trait, including comparative analysis of populations of this pathogen.

Dimethomorph is a translaminar fungicide possessing protective, therapeutic, and antisporulating properties. It has a prolonged action and is efficient at low concentrations (Cohen et al., 1995). It has no cross resistance with phenyamide fungicides.

Dimethomorph interferes with the synthesis of the cell wall and does not render a fungicide effect on protoplasts having no cell wall nor on the formation and movement of zoospores. Resistance to dimethomorph is polygenic and additive. Mutations of particular genes occur with a frequency of 6.3×10^{-7} and increase the lethal dose of fungicide insignificantly. The resistance level takes place only in double and multiple mutants (Bagirova et al., 2001). The resistant mutants differ by denser ramification of hyphae and by fewer zoosporangia (usually irregular in form). The appearance of mutations of resistance to dimethomorph is accompanied by lower adaptation both in vitro and in planta. The growth rate of resistance mutants on oat agar is less than the growth rate of the wild type (sensitive to dimethomorph). Slightly resistant mutants easily lose resistance, and the growth rate on the medium without fungicide is not restored. Resistant strains occur in a natural population extremely rarely.

During 1991–2004, the resistance to metalaxyl was studied in 2000 isolates of *P. infestans* from various regions of Russia. Resistance to metalaxyl was determined on oat agar medium with fungicide. Sensitivity of isolates to fungicides was estimated by the radial

Table 1. Annual dynamics of strains slightly resistant (SR) and resistant (R) to metalaxyl in Moscow oblast

Year	SR, %	R, %
1988	34	61
1991	19	65
1993	2	85
1996	13	44
1997	21	10
1998	6	3
1999	19	1
2000	9	20
2001	0	0
2003	24	2
2004	25	22

Table 2. Strains slightly resistant and resistant to metalaxyl in regions of the Russian Federation

Region	SR, %	R, %
Arkhangelsk oblast	6	0
Bryansk oblast	50	25
Vladivostok	40	50
Vladimir oblast	0	0
Vologda oblast	11	11
Ekatenrinburg	7	40
Ingushetia	24	20
Irkutsk	0	0
Kostroma oblast	0	0
Krasnoyarsk	8	0
Leningrad oblast	15	55
Mariy El	0	0
Mordovia	0	0
Moscow oblast	16	20
Murmansk oblast	8	92
Omsk	0	100
Ryazan oblast	45	0
Sakhalin, 1997	0	100
Sakhalin, 2003	18	80
North Ossetia	5	5
Stavropol Krai	38	12
Tula oblast	0	0
Khabarovsk	100	0
Chita	0	100
Yaroslavl oblast	0	0

growth rate of the colony on the medium with the fungicide and without it. The isolate was considered to be sensitive (S) if the relative growth rate of its colony on the medium with the fungicide (at a concentration of active substance of 10 μg/ml) in comparison with the medium without the fungicide was less than 0.1; slightly resistant (SR), at a relative growth rate from 0.1 to 0.4; and resistant (R), if over 0.4. The resistant strains had to grow on the medium with a concentration of active substance 100 μg/ml. The test was made in triplicate for each isolate.

After 1995, in most of the regions of Russia investigated, the strains sensitive to metalaxyl prevailed in populations of the agent of late blight. However, earlier, in the late 1980s–early 1990s in populations near Moscow, strains resistant to metalaxyl prevailed (Table 1). Structural changes in potato cultivation in the early 1990s led to a considerable decrease in the quantity of treatments of plantations with fungicides generally and with phenylamides in particular. This resulted in a gradual decrease of the part of resistant strains in most field populations. Beginning with 1993, in most field populations the strain resistant to phenylamides were absent or occurred as rare strains, the part of slightly resistant strains did not exceed 20–30%. At the same time, in farm fields in which phenylamide fungicides applied were applied, the portion of resistant strains was considerably higher. In 2000, in fields of the Shatura district, a population was found that was represented entirely by resistant strains; in a field in the Ruza district, the portion of resistant isolates was 31%; etc. Wider application of phenylamide fungicides resulted in an increase in resistant strains in 2004. A similar situation was also observed on tomato plantations. There were no differences in this respect between isolates from leaves of potato and tomato. It is interesting that the portion of resistant isolates on tomato fruits was significantly lower than on leaves. It is possible that during infestation of tomato fruits, the sensitive strains are advantageous.

The same regularities were recorded in an investigation of strains from regions of the European part of Russia. Sensitive isolates prevailed in field populations from the Stavropol territory; the Republic of Marii El; North Ossetia; Mordovia; and the Arkhansgelsk, Vologda, Vladimir, Ryazan, Tula, Yaroslavl, and Kostroma oblasts (collections of 2000–2002). Only in Murmansk, Bryansk, and Leningrad oblasts was the sum total of SR + R strains over 50% of the population (Table 2).

Thus, after a reduction in application of phenylamide preparations was the sensitive population of *P. infestans* restored in most regions of Russia. At the same time, in fields of farms using the preparation of this type, a considerable part of resistant strains is present. This makes it possible to forecast the portion of resistant isolates during an increase of industrial potato cultivation.

Table 3. Strains slightly resistant and resistant to metalaxyl from monomorphic populations whose strains all belong to the clonal lineage Sib 1

Region	SR, %	R, %
Sakhalin	0	100
Vladivostok	40	50
Chita	0	100
Irkutsk	0	0
Krasnoyarsk	8	0
Yekaterinburg	7	40
Omsk	0	100

In monoclonal populations of the Asiatic part of Russia, represented by the strains of one clonal line Sib 1 (isolated based on similarity of certain independent marker characters: mating type, mitochondrial DNA types, RG 57 hybridization test, spectrum of isoenzymes peptidase and glucose-6-phosphate isomerase), a diversity of strains towards metalaxyl is discovered (Elansky et al., 2001). Resistant strains of this clonal line prevailed in populations near Moscow in the early 1990s. The populations from Sakhalin, in the vicinities of Vladivistok, Chita, and Omsk (Table 3), were represented only by resistant strains. At the same time, no resistant isolates were found in populations of the same clonal lineage from the vicinities of Irkutsk. In populations from the vicinities of Krasnoyarsk and Yekaterinburg, both resistant and sensitive strains were found in an approximately equal ratio. This demonstrates that the loss and acquisition of resistance in Siberian and Far Eastern populations occurs due to mutations but not due to displacement of the strains of clonal lineage Sib 1 by a newly appearing strain with other genotypes.

In Sakhalin, a repeat survey was carried out in 2003. According to communications of specialists of the Plant Protection Service in recent years, phenylamide preparations have not been applied there. However, a high level of resistance of *P. infestans* recorded as early as 1997 has been retained until now without any change. It is remarkable that in the Murmansk and Sakhalin populations, highly resistant isolates (R > SR) quantitatively prevail among the resistant isolates. We believe that the appearance of abundant IR isolates would point to a process of reversion in the population. This was observed, e.g., in the Moscow population after the application of phenylamide preparations was reduced. It is possible that the resistant strains of *P. infestans* in Murmansk oblast and Sakhalin are characterized by a high competitive capacity that enables them to dominate in a regional population for a long time.

Great differences between the parts of resistant and sensitive isolates are observed between populations of *P. infestans* in large commercial fields and in private kitchen gardens (private plots). Resistant and slightly resistant strains occur in private kitchen gardens much more rarely than in commercial fields (Tables 4, 5). This may be related to the fact that Ridomil MC was not supplied in small parcels and until now has not been applied in private kitchen gardens. Taking into consideration that private kitchen gardens produce over 92% of the potato yield in Russia, private kitchen gardens

Table 4. Resistance to metalaxyl of strains in regions of the Russian Federation (isolation from large fields)

Region	SR, %	R, %
Odintsovo, 1993	3	97
Serebryanye Prudy, 2000	46	0
Shchelkovo raion, 2000	0	14
Ruza raion, 2000	6	31
Odintsovo, 2000	22	25
Vologda oblast, 2000	11	11
Mariy El, 2000	0	0
Kolomna raion, 2001	0	0
Tula oblast, 2001	0	0
Bryansk oblast, 2001	50	25
Leningrad oblast, 2001	15	55
Murmansk oblast, 2001	8	92
Kislovodsk, 2001	46	16
Ingushetia, 2002	24	20
Leningrad oblast, 2002	7	0
Average	15.9	25.7

Table 5. Resistance to metalaxyl of strains in regions of the Russian Federation (isolation from private plots)

Region	SR, %	R, %
Zvenigorod, 1993	13	0
Voskresensk, 1993	0	13
Zvenigorod Biostation, 1997	18	2
Glubokoe, 1997	22	7
Odintsovo, 1998	6	0
Shikhovo, 1998	6	6
Odintsovo, 1999	3	0
Moscow, 1999	25	0
Kolyubakino, 1999	38	0
Podolsk raion, 2000	0	0
Mozhaisk raion, 2000	0	0
Dubna, 2000	0	0
Chekhov raion, 2000	14	0
Shakovskoi raion, 2000	10	0
Average	11.1	2.0

Table 6. Growth of isolated on media with different dimethomorph concentrations (the number of isolates)

Region (oblast)	Total number of isolates	Growth on 0.5 μg/ml	Growth on 1 μg/ml	Growth on 2 μg/ml
Moscow	184	25	5	1 (1993)
Tula	10	0	0	0
Kostroma	11	4	0	0
Bryansk	10	2	1	0
Vologda	10	2	0	0
Leningrad	6	1	0	0
Ryazan	10	6	2	0
Murmansk	10	0	0	0
Stavropol Krai	9	5	2	0
Mordovia	10	1	0	0
Ingushetia	4	0	0	0
Omsk	20	0	0	0
Khabarovsk Krai	25	0	0	0
Sakhalin	60	5	0	0

may be considered a significant source of sensitive genotypes.

Sensitivity to dimethomorph has been investigated in 379 isolates of *P. infestans* isolated in 1993–2003 from potato plants infested with late blight in 12 regions of Russia. The experiments comprised isolates from potato plantations in Sakhalin where the preparation Acrobat-MC, containing dimethomorph, was actively applied. Agar oat medium with dimethomorph contents of 0.5, 1, and 2 μg/ml was used for testing. The sensitivity of isolates was estimated by the level at which the growth of colonies of isolates of *P. infestans* was inhibited, in comparison with the medium containing no fungicide.

The results demonstrate that development of most isolates was inhibited to a great extent already at a dimethomorph concentration in the medium of 0.5 μg/ml. A few isolates grew at a dimethomorph concentration of 1 μg/ml. However, the size of such colonies was within 15–40% of the control. One isolate grew at a concentration of 2 μg/ml (Table 6). Thus, in the investigated regional populations, no isolates resistant to dimethomorph were found.

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