

## Phenotypic characteristics of North-West Russian populations of *Phytophthora infestans* (2003-2008)

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### SUMMARY

Frequencies of *Phytophthora infestans* genes for virulence had been examined for the samples collected in North-West Region of Russia in 2003-2008. The total of 459 isolates have been collected in seven districts of St. Petersburg where the populations of *P. infestans* are very complex and possess high phenotypic diversity. Virulence to resistance genes R1 –R11 was tested using set of Black's differentials. It was detected that the mean numbers for the virulence genes per isolate increased from 7.7 in 2003 to 8.1 in 2008. The most commonly detected genes for virulence were R1 and R3. The lowest frequency was observed in gene for virulence 9 which was detected only in epidemic seasons of 2003 and 2008. The frequencies of genes for virulence R2, R5 and R6 were lower than that of other genes, which were defined in isolates collected. The analyses of isolate subgroups sampled from cultivar Newsky in 2003-2006 showed that the proportion for the number of isolates where genes for virulence R2 and R6 have not been detected was very similar. The significant difference ( $p < 0.0001$ ) was found in genes for virulence frequency in isolates sampled during two years (2004 and 2005) in the same districts from leaves of potato cultivars Snegir and Nevsky.

### INTRODUCTION

*Phytophthora infestans* is the causal agent of late blight, which is the most devastating disease in potato worldwide. In the European Union almost 6 Mha of potatoes are grown representing a value of close to €6,000,000,000. The direct and indirect costs of Late blight (costs of control and damage) estimated at more than €1,000,000,000 per one year (Haverkort *et al.* 2008). More pathogenic isolates appeared in Europe when the old clonal lineage of *P. infestans* was replaced by new more diverse population in 1990s (Goodwin and Drenth, 1977). The occurrence of A2 mating type led to sexual reproduction of *P. infestans*. Many studies conducted in potato production worldwide discovered a large spectrum of isolates with complex races (Drenth *et al.* 1994, Flier *et al.* 2003, Forbes *et al.* 1997). Both mating types had been detected in *P. infestans* populations in St. Petersburg Region in the late 1990s (Vedenyapina *et al.*, 2002). Analyses of phenotypic structure of *P. infestans* populations in North-Western Russia in two epidemic seasons (1998 and 2003) reflected that the average numbers of virulence genes per isolate increased significantly (Zoteyeva, Patrikееva, 2008). The main goal of the performed evaluation was to characterize *P. infestans* populations from St. Petersburg Region and to detect changes in genes for virulence (R1-R11) frequencies. This is important considering that frequent occurrence of genes for virulence in *P. infestans* populations increases the risk of destruction of potato plants.

## MATERIALS AND METHODS

Isolates were collected from fields of small-scale farms, experimental fields of All-Russian Institute of Plant Industry, All-Russian Institute of Plant Protection, North-West Institute of Agricultural Research and from several private seed production fields (Table 1). Fungicide treatments have not been frequently applied in majority of these fields, especially in small-scale farms. Two to seven sets were inspected in different years (Table 1).

The main goal of the research was to characterize the *P. infestans* isolates by virulence phenotypes. Four hundred fifty nine isolates were tested in total. *P. infestans* isolates were sampled from the leaflets with single lesions from the beginning of *P. infestans* manifestation until the end of vegetative period. The fragments of the infected leaflets were placed into the tuber slices of potato cultivars without R-genes. The isolates were maintained on tuber slices of susceptible cultivars (Priekulskij Rannij and Lotona) placed in glass Petri dishes (6 days of incubation, at 17-18 C°). Mating types were identified in 23 isolates collected in 2003, 2004 and 2007.

Virulence to 11 Black's R-gene differential set R1 – R11 (Black *et al.*, 1953; Malcomson & Black, 1966) each possessing a single R-gene from *Solanum demissum* was defined in *P. infestans* isolates in detached leaflet assays. Differential set was made available by IHAR-Mlochow Research Center (Poland). Two replications of three leaflets of each differential genotype were inoculated using isolates tested with 20 µl drop of sporangial suspension (50 sporangia/ mm<sup>3</sup>). The mating type tests were done by co-growth of isolates to be tested and A1 or A2 tester strains in Petri dishes with rye agar.

**Table 1.** *P. infestans* isolates sampled in 2003-2008

Year	Number of sampled isolates	Sources	Localities
2003	88	14 potato cultivars (p. cvs), 3 interspecific hybrids, 3 potato species	7 (Vyborg, Pushkin, Tosno, Gatzcina, Luga, Vsevolozhsk)
2004	69	7 p. cvs, 3 potato species	3 (Pushkin, Tosno, Gatzcina)
2005	145	4 p. cvs, 1 potato species, 1 tomato cv.	3 (Pushkin, Tosno, Gatzcina)
2006	52	3 p. cvs	4 (Pushkin, Gatzcina, Luga, Vsevolozhsk)
2007	54	2 p. cvs, 1 interspecific hybrid, 1 potato species	3 (Pushkin, Gatzcina, Luga)
2008	51	10 p. cvs	3 (Pushkin, Gatzcina, Luga)

## RESULTS AND DISCUSSION

As it was expected, the first *P. infestans* manifestation in St. Petersburg Region usually occurred in the last days of June - first days of July. The seasons 2003 and 2008 were epidemic. In 2006 and 2007 *P. infestans* manifested later, these seasons were characterized by much lowest infection spread in the first part of July in comparison with other 2003-2008 seasons. Isolates tested for mating types in 2003, 2004 and 2007 contained the both types (A1 and A2).

The numbers of pathotypes identified during the period of evaluation were: 17 in 2003, 16 in 2004, 17 in 2005, 6 in 2006, 3 in 2007 and 4 in 2008. In both 2003 and 2004 years the most frequent pathotype was 1.2.3.4.5.6.7.8.10.11. – 24% and 40%, respectively. The largest shares of other isolates from 2003 were represented by races: 1.2.3.4.6.7.8.10.11. (11%) and 1.3.4.10.11. (10%). Thirty five percent of pathotypes sampled in 2004 were of race 1.3.4.5.7.8.10.11. In the next three seasons the share with pathotype 1.2.3.4.5.6.7.8.10.11. increased to 58% in 2005, to 78% in 2006 and to 82% in 2007. In 2005 the following pathotypes were also detected: 1.3.4.5.7.8.10.11. (14%),

1.3.4.5.6.7.8.10.11. (7%), 1.2.3.4.5.7.8.10.11. (6%), 1.2.3.4.10.11. (4%) and 12 different pathotypes represented by single isolates. As it is mentioned above in 2006 and 2007 the largest shares of isolates were represented by race 1.2.3.4.5.6.7.8.10.11. In epidemic 2008 the share of this pathotype decreased to 13%, the predominant race was 1.2.3.4.6.7.8.10.11. (71%).

The overall evaluation reflected that potato late blight populations in St. Petersburg Region are very complex. The average number of virulence factors per isolate in 2003-2008 is increasing in time, particularly the number of virulence factors in late 1990s was 6.3 compare to 7.7 in 2003 and to 8.1 in 2008. In late 1990s genes for virulence 5 and 8 have been rarely detected in the collected isolates and the gene for virulence 9 was not detected at all (Vedenjapina *et al.* 2002). The frequency of gene for virulence 8 was highest in 2003 (63%) and in 2007 (100%). The higher number of genes for virulence per isolate found in 2003-2008 in comparison with data obtained in late 1990s indicates changes in phenotypic structures of late blight populations in this region. Due to the detection of A2 mating type the sexual reproduction in *P. infestans* populations can be assumed.

In all isolates collected during 2003-2008 genes for virulence R1 and R3 were common for 100% of the isolates. Genes for virulence 2, 5, 6 and 8 were less frequent. The lowest frequency was noted for gene for virulence 9. In 2003 and 2008 the frequencies of genes for virulence 5 and 8 was significantly lower then in the other seasons. The numbers of detected genes for virulence 2 and 6 during full period of evaluation were relatively close. The data obtained for isolates collected from cultivar Nevsky in 2003 - 2006 showed a positive correlation between numbers of isolate with/without these genes for virulence. It is possible to assume that cultivar Nevsky possess the ability for phenotypic selection in *P. infestans* isolates due to significant differences ( $p < 0.001$ ) in genes for virulence frequency in isolates sampled during two years (2004 and 2005) from leaves of two cultivars: Snegir (47 isolates) and Nevsky (68 isolates) (Fig.1).

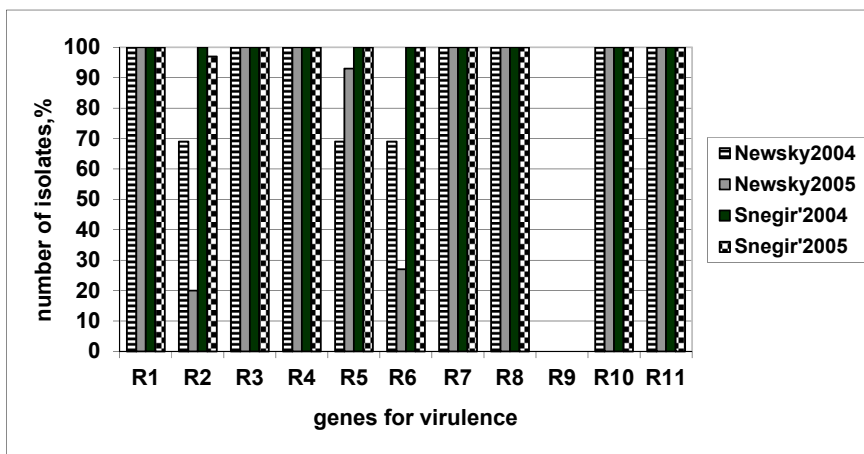


Fig. 1. Genes for virulence (R1 – R11) expression in *Phytophthora infestans* isolates sampled from leaves of potato cultivars Newsky and Snegir in 2004, 2005

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