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Distribution of mating types, metalaxyl sensitivity and virulence races of *Phytophthora infestans* in Estonia

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Abstract. Potato late blight, caused by the oomycete *Phytophthora infestans*, is a destructive potato disease, causing considerable crop loss worldwide. As the late blight pathogen population is diverse and variable in Estonia, changes in the population should be monitored regularly. In this study, the Estonian population of *P. infestans* was characterised with mating type, sensitivity to metalaxyl and virulence on potato R-gene differentials. During the growing season 2013, 110 isolates were collected from nine potato fields. The frequency of A2 mating type was on average 29%, and varied significantly between different fields from 7% to 78% ($p = 0.001$). On all studied potato fields, both mating types were recorded, suggesting continuous sexual reproduction of *P. infestans* and possible risk of oospore production and early attacks of late blight in Estonian potato fields. The prevalence of metalaxyl sensitive isolates in the population (64%) differed from results from previous research. Thus changes have occurred in the *P. infestans* Estonian population. There were no significant differences in metalaxyl sensitivity between studied fields ($p = 0.073$). The Estonian race structure was highly diverse and complex, on average 7.2 virulence factors per isolate, but varied between fields from 5.6 to 9.0. 42 virulence races were found; the four most common were 1.2.3.4.5.6.7.8.10.11, 1.2.3.4.6.7.8.10.11, 1.2.3.4.7.10.11 and 1.2.3.4.7.8.10.11, which comprised 46% of the population. The overall normalized Shannon's diversity index was 0.69, confirming the high diversity of the population. Continuous pathogen population studies describing the contemporary *P. infestans* population are essential in order to advise potato breeders and growers accordingly.

Key words: mating type, metalaxyl, *Phytophthora infestans*, population variation, potato late blight, virulence testing.

INTRODUCTION

Potato late blight is caused by the oomycete pathogen *Phytophthora infestans*; it first appeared in Europe more than 160 years ago but remains a major threat to potato crops both in Europe and worldwide. Despite recent active research and progress, late blight still requires vigilance and often numerous applications of fungicide for effective control (Cooke et al., 2011). It is a serious problem for Estonian potato production, particularly under favourable conditions, when it can destroy the whole potato haulm and the average loss due to late blight can reach 20–25% and in untreated fields even more (Runno-Paurson et al., 2010). Fungicides are used routinely in conventional potato production, but under favourable conditions for the disease, with heavy pressure from the pathogen, protection of large areas is complicated (Runno-Paurson et al., 2010).

Potato late blight pathogen *P. infestans* is an heterothallic organism with two mating types A1 and A2. The pathogen is able to reproduce sexually and asexually. During the 1980s, *P. infestans* populations containing both mating types migrated from Mexico apparently in 1976/77 into Europe giving rise to sexually reproducing populations (Fry & Goodwin, 1997). New diverse *P. infestans* genotypes were very adaptable and spread quickly all over Europe displacing the old clonal lineage which is now found only rarely (Carlisle et al., 2002; Cooke et al., 2011). *P. infestans* benefits from sexual reproduction by increased adaptability of the pathogen and production of oospores that can survive in the soil for several years (Turkensteen et al., 2000; Yuen & Andersson, 2013). While low temperature could even conserve the viability of the oospores (Turkensteen et al., 2000; Hannukkala, 2012) the pathogen benefits from it in Estonia during cold winters. Furthermore, genotyping *P. infestans* Estonian isolates with SSR markers revealed high genetic diversity and provided evidence that sexual reproduction and recombination are common in this population (Runno-Paurson et al., 2016).

While the late blight pathogen population is diverse and variable in Estonia, changes in the population should be monitored regularly. Therefore, in this study, potato late blight pathogen *Phytophthora infestans* isolates collected in 2013 from different potato fields in Estonia were characterised with phenotypic characteristics such as mating type, metalaxyl sensitivity and virulence to Black's differentials.

MATERIALS AND METHODS

Collection and isolation of *P. infestans* strains

Potato leaves infected by *P. infestan*s were collected in 2013 from nine sites from five counties in Estonia (Table 1). The samples were taken from large scale conventional and small scale conventional growers' potato fields, potato field trials and an organic field (Table 1). In large scale conventional productions farmers used high-quality certified seed potatoes and applied fungicide 6–8 times per season. The metalaxyl-based fungicide Ridomil Gold MZ 68 WG was applied at the beginning of disease infection at least twice. Rotation varied between 0–2 years on these farms. Small scale conventional farmers used uncertified seed potatoes and no fungicides routinely for late blight control. The interval between growing potato crops was 1–2 years. At the potato field trials in Reola and at the Estonian Crop Research Institute in Jõgeva fungicide was not used, but at Lepiku field trial fungicides were applied three times per season. At the organic field and field trials growers rotated fields and grew potatoes every 3–4 years.

Nine to fifteen isolates were cultured from each sampling site (Table 1). The sampled plants were located at a random distance from field edges. Only single lesion leaves were collected from each plant, taken randomly; any leaves with several or no lesions were excluded. Isolations were carried out and maintained using methods described by Runno-Paurson et al. (2009). All phenotypic tests were carried out immediately after the isolations were finished (October to January). *P. infestans* isolates of this study are preserved at Tartu Fungal Collection (TFC).

Table 1. The number of *Phytophthora infestans* isolates collected in 2013 and tested for mating type, metalaxyl sensitivity and virulence phenotype

Sampling site	County	Crop type*	Tested for		
			Mating type (n)	Metalaxyl (n)	Virulence (n)
Jõgeva 1	Jõgeva	Trial field	12	12	12
Jõgeva 2	Jõgeva	Organic	14	14	14
Antsla	Võru	LSC	14	14	13
Lepiku	Tartu	Trial field	15	15	13
Reola	Tartu	Trial field	11	11	11
Sürgavere	Viljandi	LSC	9	9	8
Tilga	Tartu	SSC	11	11	11
Verioramõisa	Põlva	LSC	14	14	14
Võnnu	Tartu	SSC	10	10	8
Total			110	110	104

* - LSC (large scale conventional), SSC (small scale conventional).

Phenotypic analyses

Mating types were determined by the method described in Runno-Paurson et al. (2009). The tester isolates were 90209 (A1) and 88055 (A2) as described in Hermansen et al. (2000). Isolates forming oospores on plates with the A1 mating type were registered as A2; isolates that formed oospores with the A2 mating type were registered as A1.

P. infestans isolates resistance to metalaxyl was tested using a modification of the floating leaflet method (Hermansen et al., 2000). Leaf disks (14 mm diameter) were cut with a cork borer from leaves of five-week-old greenhouse-grown potato plants. The susceptible cultivar 'Berber' was used. Six leaf disks were floated abaxial side up in Petri plates (50 mm diameter) each containing 7 mL distilled water or metalaxyl in concentrations of 10.0 or 100.0 mg L^{-1} prepared from technical grade metalaxyl-M (Syngenta experimental compound (metalaxyl-M), CGA 329351A). The inoculation and trial incubation was done as described by Runno-Paurson et al. (2009). The isolates were rated resistant if they sporulated on leaf disks in 100 mg L^{-1} metalaxyl (Hermansen et al., 2000). Those sporulating on leaf disks in a metalaxyl concentration of 10 mg L^{-1}, but not on leaves floating on 100 mg L^{-1} were rated intermediate, and those sporulating only in water were rated sensitive.

The virulence pathotype was determined with detached leaflet set of Black's differentials of potato genotypes containing resistance genes R1–R11 from *Solanum demissum* (Malcolmson & Black, 1966) (provided by the Scottish Agricultural Science Agency). Laboratory procedures were as described in Runno-Paurson et al. (2009).

Data analysis

Statistical analyses were performed with the SAS/STAT version 9.1 (SAS Institute Inc., Cary, NC, USA). Differences in the prevalence of the two mating types of *P. infestans* isolates between study sites were tested using a logistic analysis (GENMOD procedure in SAS) with a multinomial response variable (A1, A2, or both). Analogous logistic procedures were used to examine the differences in the resistance to metalaxyl (a multinomial response variable: resistant, intermediate or sensitive) between sites and also between different mating types. The dependence of specific virulence (percent of isolates that show virulence against particular R-genes) on site and R-genes was analysed

with type III ANOVA and Tukey HSD post-hoc tests ($\alpha = 0.05$). In all analyses, 'site' was treated as a categorical variable.

Race diversity was calculated with the normalized Shannon diversity index (Sheldon, 1969). The differences in the Shannon index values between sites was analysed with one-way ANOVA and Tukey HSD test.

RESULTS AND DISCUSSION

Both A1 and A2 mating types were found among *P. infestans* isolates collected in Estonia in 2013. Out of 110 tested isolates, 71% were classified as A1 mating type and 29% were classified as A2 mating type (Table 2). There were considerable differences in the proportion of A1 and A2 between sampling sites (*Chi-square* = 25.44, *df* = 8, $p = 0.001$), with the frequency of A2 mating type varying between different potato fields from 7 to 78% (Table 2).

Table 2. Percentages of mating types among isolates of *Phytophthora infestans* in Estonia in 2013

Site	Mating type (%)		Number of isolates
	A1	A2	
Jõgeva 1	75	25	12
Jõgeva 2	71	29	14
Antsla	43	57	14
Lepiku	93	7	15
Reola	91	9	11
Sürgavere	22	78	9
Tilga	73	27	11
Verioramõisa	93	7	14
Võnnu	60	40	10
Total	71 ± 7.6*	29 ± 7.6*	110

* – mean ± SE.

The average percentage of A2 mating type was a bit lower than previously recorded in Estonia (Runno-Paurson et al., 2010; Runno-Paurson et al., 2013; Runno-Paurson et al., 2014), but temporal fluctuation has been noticed before (Runno-Paurson et al., 2012). However, our findings on mating types in the Estonian population are generally comparable with populations described recently in studies from the Nordic countries Finland, Denmark, Norway and Sweden (Lehtinen et al., 2008; Hannukkala, 2012), Latvia (Aav et al., 2015), Lithuania (Runno-Paurson et al., 2015), Poland (Chmielarz et al., 2014) and the north-western part of Russia (Statsyuk et al., 2013). In this study both mating types were recorded from all studied potato fields (Table 2), indicating continuous sexual reproduction of *P. infestans* and possible risk of oospore production and early attacks of late blight in Estonian potato fields. As a result of sexual reproduction high genetic diversity in the population can also be expected as shown in the previous *P. infestans* collection from 2004 characterized with SSR markers (Runno-Paurson et al., 2016).

Of the 110 isolates tested for metalaxyl response, 64% were sensitive, 20% were intermediate and 16% were resistant to metalaxyl (Table 3). Significant differences were not found between sampling sites (*Chi-square* = 24.83, *df* = 16, $p = 0.07$).

Table 3. Metalaxyl sensitivity among isolates of *Phytophthora infestans* from Estonia in 2013

	Percentage of isolates			Number of isolates
	S*	I*	R*	
Jõgeva 1	75	8	17	12
Jõgeva 2	57	7	36	14
Antsla	64	36	0	14
Lepiku	67	20	13	15
Reola	100	0	0	11
Sürgavere	78	11	11	9
Tilga	55	36	9	11
Verioramõisa	36	36	28	14
Võnnu	50	20	30	10
Total	64 ± 5.8**	20 ± 4.4**	16 ± 4.1**	110

* – S, metalaxyl sensitive; I, intermediate metalaxyl sensitive; R, metalaxyl resistant;
** – mean ± SE.

The prevalence of metalaxyl sensitive isolates in the population showed clearly different results compared to previous research done in Estonia (Runno-Paurson et al., 2010; Runno-Paurson et al., 2014). This suggests changes in the *P. infestans* Estonian population. These results are similar to recent findings from Latvia (Aav et al., 2015), Lithuania (Runno-Paurson et al., 2015), Poland (Chmielarz et al., 2014) and other European populations of *P. infestans*. The significant association between response to metalaxyl and mating type was not found (*Chi-square* = 1.11, *df* = 2, *p* = 0.57).

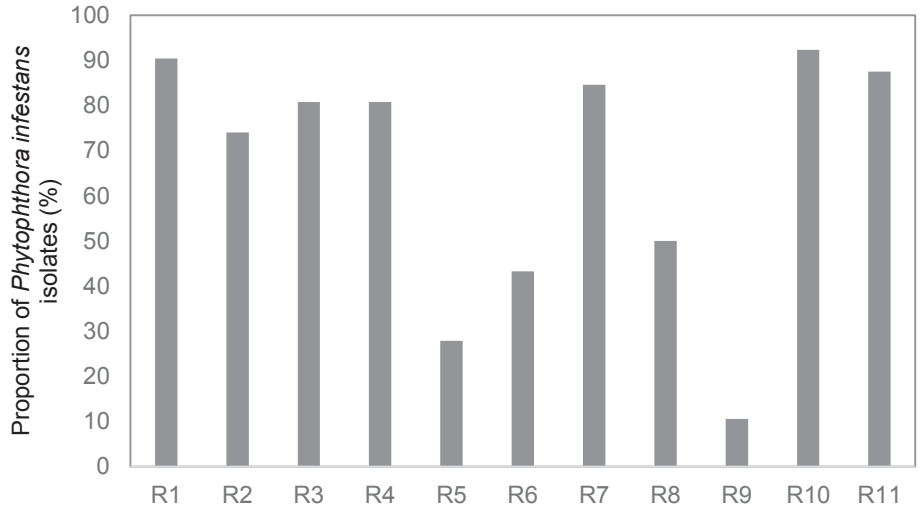

Figure 1. Frequency of virulence to potato R-genes in the Estonian population of *Phytophthora infestans* in 2013.

All 11 known virulence factors were found among the 104 *P. infestans* isolates tested for virulence (Fig. 1). Almost all isolates were virulent on differentials with genotypes R1, R2, R3, R4, R7, R10 and R11. Virulence factors 9 (11%) and 5 (28%) were relatively rare (Fig. 1). No significant differences in virulence factors were found between field sites ($F_{(8,80)} = 2.64$, $p = 0.11$). The Estonian race structure was highly

complex, on average 7.2 virulence factors per isolate, but varied between fields from 5.6 to 9.0. The overall normalized Shannon's diversity index was 0.69 and varied between potato fields from 0.43 to 0.95 (Table 4), confirming the high diversity of the population. 42 virulence races were found and the four most common virulence races were 1.2.3.4.5.6.7.8.10.11, 1.2.3.4.6.7.8.10.11, 1.2.3.4.7.10.11 and 1.2.3.4.7.8.10.11, comprising 46% of the population (Table 5). Twenty-six races were unique and found only once (Table 5).

Table 4. Racial diversity of *Phytophthora infestans* from different sites in Estonia in 2013

Site	Hs*
Jõgeva 1	0.43
Jõgeva 2	0.87
Antsla	0.75
Lepiku	0.72
Reola	0.58
Sürgavere	0.75
Tilga	0.95
Verioramõisa	0.80
Võnnu	0.92
Total	0.69

* – the normalized Shannon diversity index for race diversity calculation.

Table 5. Race frequencies among isolates of *Phytophthora infestans* from Estonia in 2013

Races	Number of virulence factors	Number of isolates
1.2.3.4.5.6.7.8.10.11	10	16
1.2.3.4.5.6.7.9.10.11	10	3
1.2.3.4.5.6.7.10.11	10	3
1.2.3.4.6.7.8.10.11	9	11
1.2.3.4.6.7.9.10.11	9	3
1.2.3.4.5.7.8.10.11	9	2
1.2.3.4.7.8.10.11	8	10
1.2.3.4.6.7.10.11	8	3
1.2.3.4.5.7.10.11	8	2
1.2.3.4.7.10.11	7	11
1.3.4.7.8.10.11	7	2
1.3.4.7.10.11	6	4
1.4.7.10.11	5	2
1.7.10	3	2
1.10	2	2
10	1	2
Races found once		26
Total number of isolates		104
Total number of races		42

The frequencies of virulence factors in this study were similar to those reported recently in Estonia (Runno-Paurson et al., 2009; Runno-Paurson et al., 2010; Runno-Paurson et al., 2014), except for virulence factor 2, the frequency of which has increased over the years. The prevailing race 1.3.4.7.10.11 of *P. infestans* in most European populations (Hermansen et al., 2000; Lehtinen et al., 2008; Hannukkala, 2012; Chmielarz et al., 2014; Runno-Paurson et al., 2014) was found only four times (3.8%) from the Estonian population. The average number of virulence factors per isolate was 7.2, which is quite similar to that found in other populations from Estonia in previous long-term studies (Runno-Paurson et al., 2012; Runno-Paurson et al., 2014) and also among Eastern European populations (Śliwka et al., 2006; Statsyuk et al., 2013; Aav et al., 2015; Runno-Paurson et al., 2015).

CONCLUSIONS

In this study both mating types of *P. infestans* were recorded in all studied potato fields, indicating continuous sexual reproduction and possible risk of oospore initiated early attacks of late blight in these fields. As metalaxyl-sensitive isolates dominated in the pathogen population sensible and moderate use of metalaxyl based fungicides in most fields could be employed. Overall, the *P. infestans* population in Estonia is highly diverse and complex, characterised by high virulence race diversity. Continuous pathogen population studies describing the contemporary *P. infestans* population are essential in order to advise potato breeders and growers accordingly.

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