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Impact of Phytosanitary Quality of Seed Potato and Temporal Epidemic Progress on the Phenotypic Diversity of *Phytophthora infestans* Populations

Eve Runno-Paurson · Asko O. Hannukkala · Katrin Kotkas · Mati Koppel · Ingrid H. Williams · Marika Mänd

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Abstract Altogether 365 isolates of *Phytophthora infestans* were sampled from potatoes propagated from seed potatoes of high (multiplied for two years in open field after meristem phase) and low (commercial certified seed multiplied for several consecutive years in open field) phytosanitary quality at different phases of epidemic progress during the growing seasons of 2001–2007 from field plots at two experimental institutes in Estonia, North-East Europe. High or low phytosanitary quality of seed potatoes had no effect on mating type ratio or response to metalaxyl in populations of *P. infestans* isolated from these two different groups of potato material. In contrast, the incidence of certain virulence factors, as well as the diversity of pathotypes, was very high in populations collected from potatoes propagated from low-quality seed in comparison to those from high-quality seed. The incidence of A2 mating type and fully metalaxyl sensitive strains was statistically significantly higher at the epidemic outbreak than later during epidemic progress. The incidence of most virulence factors and overall pathotype diversity were not affected by the temporal progress of the epidemic. Rare virulence factors 5 and 9 were more frequent at the outbreak of the epidemic and declined in the population during the course of epidemic.

Resumen En total, se obtuvieron 365 aislamientos de *Phytophthora infestans* de papas propagadas por tubérculo-semilla de alta (multiplicada por dos años en campo abierto después de la fase de meristemo) y baja (semilla certificada comercial multiplicada por varios años consecutivos en campo abierto) calidad fitosanitaria, a diferentes fases de progreso de la epidemia durante los ciclos de cultivo de 2001 a 2007 de lotes de campo en dos institutos experimentales en Estonia, Europa del Noreste. La calidad fitosanitaria alta o baja de la semilla de papa no tuvo efecto en la proporción de los grupos de compatibilidad o en la respuesta al metalaxil en poblaciones de *P. infestans* aisladas de estos dos diferentes grupos de material de papa. En contraste, la incidencia de ciertos factores de virulencia, así como la diversidad de patotipos, fue muy alta en poblaciones colectadas de papas propagadas de semilla de baja calidad en comparación con aquellas de semilla de alta calidad. La incidencia de variantes del grupo de compatibilidad A2 y completamente sensibles a metalaxil fue más alta con significancia estadística al inicio de la epidemia que más tarde, durante el progreso epidémico. La incidencia de factores de mayor virulencia y la diversidad de patotipo en general no se afectaron por el progreso temporal de la epidemia. Los factores de virulencia raros 5 y 9 fueron más frecuentes al estallamiento de la epidemia y declinaron en la población durante el transcurso de la misma.

Keywords *Phytophthora infestans* · Mating type · Virulence · Pathotype · Potato cultivars

E. Runno-Paurson (✉) · I. H. Williams · M. Mänd
Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51014, Estonia
e-mail: eve.runno-paurson@emu.ee

A. O. Hannukkala
MTT Agrifood Research Finland Plant Production Research, 31600 Jokioinen, Finland

K. Kotkas
Department of Plant Biotechnology EVIKA of Estonian Research Institute of Agriculture, Teaduse 6a, Saku 75501 Harjumaa, Estonia

M. Koppel
Department of Biochemistry and Plant Protection, Jõgeva Plant Breeding Institute, Jõgeva alevik 48309, Estonia

Introduction

The most important disease in potato is potato late blight caused by the oomycete *Phytophthora infestans* (Mont.) de Bary. In North-East Europe, under favorable cool and moist conditions, the pathogen can cause considerable yield loss when susceptible potato cultivars are grown without chemical protection. In Nordic countries, during the last decade, there have been indications of earlier infections of *P. infestans* and, for that reason, more frequent fungicide treatments have been needed to control the disease (Hannukkala et al. 2007). Data from Finland and Estonia show that the first findings of late blight now occur one month earlier than 20 years ago and blight infections are more severe (Hannukkala et al. 2007; Koppel and Runno 2006).To protect foliage and tubers and also to prevent damage, growers in North and West Europe have to apply fungicides almost weekly. The chemicals are an environmental hazard and excessive use of pesticides may reduce the perception of the potato as a healthy food (Haverkort et al. 2008).

After the migration of the new population of *Phytophthora infestans* during the last two decades, potato late blight has caused increasing problems in Europe (Cooke et al. 2011). Sexual reproduction has changed the ecology of *P. infestans*. Nowadays, it reproduces sexually in most European countries (Cooke et al. 2011) and the frequency of the mating types in European populations has changed in recent years; several studies have reported a rapid increase in the proportion of the A2 mating type (Hermansen et al. 2000; Śliwka et al. 2006; Lebecka et al. 2007; Lehtinen et al. 2007; Runno-Paurson et al. 2009; Chmielarz et al. 2010; Kildea et al. 2010; Runno-Paurson et al. 2010a, b).

The R-gene pathotype structure of the late blight population is highly diverse and complex (Śliwka et al. 2006; Runno-Paurson et al. 2009; Zoteyeva and Patrikeeva 2010). Hannukkala et al. 2007 have indicated that oospores, as a soil-borne inoculum, are not the only cause for early attacks of late blight, but climate change also has a role. One possible source of high phenotypic diversity is contaminated seed potatoes. The phytosanitary quality of seed potatoes influences late blight epidemics. Cropping system-specific differences appeared in the population of *P. infestans*, which arose from different management practices including seed source and foliar resistance (Runno-Paurson et al. 2010b).

The first step in integrated control is to reduce the primary source of inoculum (Cooke et al. 2011). In Western Europe, the main late blight inoculum source is overwintering tubers from dumps, volunteer plants and infected seed tubers (Evenhuis et al. 2007; Cooke et al. 2011). However, in North European regions, primary infections from overwintering infected tubers are less important than in regions with milder winters and infected seed tubers together with oospores are the most important primary infection sources (Lehtinen & Hannukkala, 2004; Widmark et al. 2007). Kuznetsova et al. 2010 have reported on the overwintering of oospores on tomatoes in North–West Russia and found that *P. infestans* oospores can overwinter in the soil and, at least in the course of the next season, be a source of infection, causing the appearance of the first late blight lesions on plants. In Baltic countries, oospore overwintering has been not studied, but in several reports from Estonia (Runno-Paurson et al. 2009, 2010a, 2011) where both mating types (A1, A2) co-existed in most sampled potato fields, that oospores may have quite an important role for pathogen over wintering and cause earlier late blight outbreaks in Estonia. Because of oospores, crop rotation is now a part of integrated late blight control in Europe (Cooke et al. 2011).

Historically, in Estonia, potatoes have been grown on allotments and on small field areas (up to 0.3 ha) in continuous monoculture for decades on the same field from the same potato source. In 2011, potatoes were grown on 9,300 ha in Estonia, of which 2500 ha are estimated to have been grown on allotments and small growers' fields. These home-farms grow potatoes continuously in the same field plot using their own multiplied seed material which is usually a mix of different cultivars. This kind of system does not exist in the rest of Europe but is common in Eastern Europe. However, in Estonia, as well as the oospore infection, these allotments are the main initial and on-going source of late blight inoculum throughout the growing season, dispersing inoculum to large potato seed and production fields.

The aim of this study was to investigate whether the high phytosanitary quality of seed potato obtained by recent meristem propagation reduces the phenotypic diversity of the Estonian late blight population in comparison to seed material grown for numerous generations in the open field, and how these traits in general fluctuate time-wise along with late blight epidemic progress.

Materials and Methods

Collection and Isolation of Isolates

During the seven consecutive years, 2001–2007, 387 isolates of *P. infestans* were collected from genetically diverse potato cultivars. Isolates were collected from potato originating from seed with high phytosanitary quality (185 isolates) propagated for 1–2 years in the open field after the meristem phase and from potato originating from seed with low phytosanitary quality (180 isolates) propagated from commercial certified seed for various generations in the open field (Table 1). In 2001, a total of 55 isolates were sampled and 53, 47, 62, 53, 46 and 71 isolates were sampled

Table 1 Number of *Phytophthora infestans* isolates characterized for their mating type, virulence factors and response to metalaxyl originating from potatoes propagated with seed with high or low phytosanitary quality at different phases of the late blight epidemic in Estonia during 2001–2007

Origin	Number of isolates tested for		
	Mating type	Virulence factors	Response to metalaxyl
Phytosanitary quality of seed potato			
High quality[a]	161	186	108
Low quality[b]	133	180	71
Phase of epidemic			
Beginning	157	180	108
Two weeks after	41	46	19
Advanced phase	94	110	44

[a] High quality seed was propagated in the open field 2 years after meristem phase

[b] Low quality seed was propagated from commercial certified seed multiplied in the open field for several consecutive years

during 2002–2007, respectively. The field plots were located at the Department of Plant Biotechnology EVIKA of the Estonian Research Institute of Agriculture in north Estonia (59°17′N, 24°37′E) and at Jõgeva Plant Breeding Institute in east Estonia (58°44′N, 26°25′E). The institutes differed in the origin of their seed material, in potato variety background and in pesticide input.

Both institutes grew a high genetic diversity of potatoes including several genotypes with race-specific R-genes, and diverse cultivars and breeding lines. At both institutes conventional agrotechnical methods were used. In EVIKA, seed potatoes shifted after two years growing meristem plants (Rosenberg and Kotkas 1986; Särekanno et al. 2010). Multiplication of potato plants is done from stem and tip cuttings as well as via truncated-meristem plants grown on plastic rolls in a peat substrate. Fungicides were used once per growing season in all years, except in 2006. At Jõgeva Plant Breeding Institute, the quality of seed potatoes was diverse, varying from healthy certified seed material, imported potato seeds from Western Europe to diseased, old seed material; fungicides were not used for late blight control.

Four to twenty-five leaflets, each with a single lesion (one per plant), were collected from all collection sites at different stages of the epidemic during most years, namely at the beginning of the epidemic, at the middle of the epidemic (1–2 weeks later) and at the end of the growing season (>3 weeks later). In addition, tuber samples were collected during the storage season in 2001. In the early stages of the outbreak, approximately 10–15 % of the leaf area of the infected plants and less than 10 % of plants were infected with late blight. In the later stages, about 20–30 % of the leaf area and more than 50 % of the plants were infected.

Isolation Technique

Isolations were carried out by placing a fragment of infected leaf tissue between tuber slices that had been sterilized by ethanol and flaming. Tubers of susceptible cultivars without known R genes were used (Berber or Bintje). The slices were put into a sterile Petri dish with a moist filter paper disk on top. The Petri dish was incubated for 6–7 days at 16 °C in a growth chamber until the mycelia had grown through the slices. A small sample of mycelia from the tuber slices was transferred with a sterile needle to rye B agar (Caten and Jinks 1968). The pure cultures were preserved at 5 °C and transferred to rye agar after every 2 months. All phenotypic tests were carried out in October–November of the year of isolation.

Phenotypic Analyses

Mating types, A1 or A2, were determined by the method described in Runno-Paurson et al. 2009. Observed oospore formation in single-isolate pure cultures was interpreted as the occurrence of self-fertility in the isolates. The tester isolates were the same as those described in Lehtinen et al. 2007.

The specific virulence of each of the 196 isolates was determined using Black's differential set of potato genotypes containing resistance genes R1–R11 (Malcolmson and Black 1966) (provided by the Scottish Agricultural Science Agency). Leaves were obtained from the differentials grown from tubers in the greenhouse or growth chamber. Fully expanded young leaflets collected from the middle part of each differential plant at 6–8 weeks of age were inoculated. Leaflets were placed abaxial surface up in trays containing moistened filter paper and each leaflet was inoculated with a 20 μL drop of sporangial suspension (1.0–4.0×10^4 sporangia ml^{-1}) prepared from 7–9 day-old cultures on rye B agar. Three leaflets per isolate were used and the trial was replicated twice. The trays were covered with polyethylene after the inoculation to maintain high humidity and were incubated at 16 °C with a 16-h photoperiod, and 8-h dark period. The interactions between the pathogen and potato genotypes were scored seven days after inoculation, using the following scale: 0, no symptoms; 1, small necrotic lesion; 2, <10 % area covered; 3, 10–50 % area covered; 4, 50–75 % area covered and 5, >75 % area covered. The reaction was compatible if sporulation was detected in at least four leaflets out of six, and the cumulative score was at least 15. Compatible interactions were usually indicated by large, sporulating lesions.

The resistance to metalaxyl of all 179 isolates was tested using a modification of the floating-leaflet method (Hermansen

et al., 2000). Leaflets of susceptible cultivars (Berber in 2001–2002; Bintje in 2003–2007) were obtained from five-week-old greenhouse-grown plants. The metalaxyl concentrations were 0.0, 10.0 or 100.0 mgL^{-1} prepared from Analytical Master Standard, CGA 48988 (Ciba Geigy, purity 99.6 % metalaxyl). The sporangia were multiplied on rye B agar and collected in distilled water with a paintbrush. Spore concentration was adjusted to 10,000 sporangia mL^{-1} and 20 μl of the suspension was placed in the centre of each leaflet floating on 0.0 mg metalaxyl L-1 or water containing metalaxyl solution, in a plastic tray. The tray was covered with polyethylene after inoculation to maintain high humidity. Inoculated leaflets were kept on plastic trays for seven days in natural daylight at 15 °C and under high relative humidity. The assessment was performed in two replicates and the whole trial was replicated twice. Four leaflets were used for each isolate-metalaxyl concentration combination. After seven days, the area covered by sporangiophores was estimated visually as a percentage of the total area of the leaflet using the same scale as indicated for the assessment of specific virulence. Sporulation was regarded as present if the cumulative score for all four leaflets was at least 12. The resistance determination was done by discriminatory dose, where isolates were rated as resistant if they sporulated on leaflets in 100 mgL^{-1} metalaxyl. Those sporulating on leaflets in a metalaxyl concentration of 10 mgL^{-1}, but not on leaves floating on 100 mgL^{-1} were rated tolerant, and those sporulating only in water were rated sensitive.

Statistical Analyses

The risks (odds ratios) for the incidence of A2 mating type, virulence factors 1 to 11 and different metalaxyl response classes in high-quality propagation material in comparison to low-quality seed material and at the early phases of epidemics in comparison to the advanced phase were calculated using a logistic regression approach as described in detail by Allison 1999.

The ‘odds’ of an event are defined as the probability of the outcome event occurring divided by the probability of the event not occurring. In general, the ‘odds ratio’ is one set of odds divided by another. An odds ratio of 1 indicates that the event under study is equally likely in both values of the predictor. An odds ratio greater than 1 indicates that the event is more likely in the first value, whilst an odds ratio of less than 1 indicates that the event is less likely in the first value. The odds ratio must be zero or greater than zero. A 95 % confidence interval for the odds ratio helps to understand how high and how low the actual population odds ratio might be. The confidence intervals are related to the *P*-values such that the odds ratio will not be statistically significant if the confidence interval contains 1. Logistic regression was used to analyze *P. infestans* isolate frequency data because it was considered more appropriate than other statistical methods, which assume that the residuals, or errors, are drawn from a normal distribution. Logistic regression has the additional advantage that all of the predictors can be binary, a mixture of categorical and continuous or just continuous. The disadvantage of the method is that predictors cannot be calculated if all the observations in a certain category get the value 0 or all are rated as 1 (Allison 1999). The use of logistic regression to analyze similar frequency data has been described in detail by Lehtinen et al. 2007. These analyses were performed by the SAS/logistic procedure in SAS/STAT version 9 (SAS Institute Inc., Cary, NC, USA).

To compare the differences in diversity of populations between different phytosanitary quality classes of seed and different phases of late blight epidemic the Shannon diversity index was calculated as follows: $HS=-\Sigma j(pj*lnpj)$, $j=1\ldots Np$, where pj is the frequency of the j th race in the population and Np is the total number of races in the population. The bias created for HS by differences in sample sizes between populations was corrected by using the relative Shannon diversity index as follows: $HSR=HS/lnNi$, where Ni is the number of individuals in the population (Goodwin et al. 1992, Andrivon 1994).

Shannon's index accounts for both abundance and evenness of the virulence pathotypes present. Values for HSR range from 0 (single pathotype present) to 1 (each isolate in the sample has a different pathotype). This statistic, presenting the Shannon index as a fraction of the maximum diversity in the sample provides a good measure when sample sizes vary (Sheldon, 1969). The dependence of virulence complexity on isolation time was analysed with one-way ANOVA and Tukey HSD test, as were the differences in the Shannon index values between isolation time and quality of seed potatoes.

Results

The *P. infestans* population collected for this study in 2001–2007 from two distinct locations in Estonia in general was diverse. The overall proportion of mating type A1 was 60 % and of A2 was 39 %. Approximately 1 % of the isolates produced oospores in the presence of both A1 and A2 tester isolates (Table 2).

All known virulence factors from 1 to 11 were found among the isolates tested. Resistance of differentials with genes R1, R3, R4, R7, R10 and R11 was overcome by 93–97 % of the isolates (Table 3). Resistance genes R2, R6 and R8 were overcome by 49, 42 and 31 % of the isolates, respectively (Table 4). Only 9 % of the isolates were able to overcome gene R5 and 11 % gene R9 (Table 5). The average number of virulence factors in all isolates was 7.1. There were 72 virulence pathotypes among the 366 isolates

Table 2 Effect of the phytosanitary quality of seed potato and the phase of potato late blight epidemic to the prevalence (%) of A2 mating type in Estonian *P. infestans* populations in 2001–2007

Origin of the *P.infestans* isolate	Prevalence of mating type A2 %	Prevalence of mating type A12 %[a]	Sample size N	Odds ratio and p-value for A2 incidence vs. control
Phytosanitary qualityof seed potato				
High quality[b]	42	1	161	1.76 p=0.0627
Low quality[c]	29	3	133	Control
Phase of epidemic				
Beginning	47	0	157	2.54 p=0.0016
Two weeks after	34	0	41	1.42 p=0.3990
Advanced phase	28	4	93	Control
Total population	60	1	294	

[a] A12 isolates were producing oospores with both A1 and A2 tester strains; their frequency is too low to be analyzed statistically

[b] High quality seed was propagated in the open field 2 years after meristem phase

[c] Low quality seed is propagated from commercial certified seed multiplied in the open field for several consecutive years

tested. Slightly more than 50 % of the population was comprised of the three most common pathotypes (1.3.4.7.10.11; 1.2.3.4.6.7.10.11 and 1.2.3.4.7.8.10.11) and 75 % of the population belonged to the 10 most common pathotypes. Almost 60 % of the different pathotypes were found only once. These figures have been calculated over all isolates and are not shown in tables (Table 6). Among 179 isolates tested for their response to metalaxyl, 35 % were classified as resistant, 36 % as tolerant and 29 % as sensitive (Table 7). Among metalaxyl-resistant strains, 53 % were A1 mating type and 47 % were A2 mating type (Odds ratio A1 vs. $A2$=0.64; p=0.2266). Within metalaxyl-tolerant isolates, 69 % belonged to A1, 31 % to A2 mating type (Odds ratio A1 vs. $A2$=1.74; p=0.1541); and 59 % of the metalaxyl sensitive isolates were A1 and 41 % A2 type (Odds ratio A1 vs. $A2$=0.92; p=0.8197). Thus, there was no statistically significant association between response to metalaxyl and mating type.

Phytosanitary Quality of Seed and Phenotypic Traits of the Blight Population

Phytosanitary quality of seed potatoes had no statistically significant effect on the mating type ratio in respective *P. infestans* populations. The proportion of the A2 mating type in isolates collected from high-quality seed was 42 % while that from low-quality seed was 29 % (Odds ratio 1.76; p=0.0627). A few isolates producing oospores with both mating type tester isolates were isolated from both seed classes (Table 2).

The influence of seed quality on the occurrence of certain virulence factors and the diversity of virulence pathotypes was extremely high. Differences in the occurrence of the almost universal virulence factors (1,3,4,7,10,11) between isolates collected from high-and low-quality seed were not statistically significant. Nevertheless, the population collected from potatoes propagated with high-quality seed was also more uniform in this respect than that from low-quality seed. Practically all isolates contained these virulence factors (Table 3). The probability for the occurrence of relatively common virulences (2,6,8) was statistically 3–5 times lower (odds ratios 0.20–0.30) in isolates collected from plants with high seed quality than in isolates originating from plants with low seed quality (Table 4). Similarly, the probability of finding virulence factor 5 was 5 times lower (odds ratio 0.21) and virulence factor 9 respectively (odds ratio 0.19) in isolates originating from high-quality seed than those from low-quality seed (Table 5).

Table 3 Effect of the phytosanitary quality of seed potato and the phase of potato late blight epidemic to the prevalence (%) of 'universal' virulence factors 1, 3, 4, 7, 10 and 11 in Estonian *P. infestans* populations in 2001–2007. Due to high frequency of each virulence factor logistic regression cannot detect any statistically significant differences between origins of isolates

Origin of P. infestans isolate	% of isolates containing virulence factor (VF)						
	VF1	VF3	VF4	VF7	VF10	VF11	Sample size N
Phytosanitary quality of seed potato							
High quality[a]	97	96	97	99	94	93	186
Low quality[b]	89	91	88	95	93	95	180
Phase of epidemic							
Beginning	94	93	94	98	96	96	180
Two weeks after	93	96	91	98	87	83	46
Advanced phase	96	97	96	98	94	93	110

[a] High quality seed was propagated in the open field 2 years after meristem phase

[b] Low quality seed is propagated from commercial certified seed multiplied in the open field for several consecutive years

Table 4 Effect of the phytosanitary quality of seed potato and the phase of potato late blight epidemic to the prevalence (%) of relatively common virulence factors 2, 6 and 8 and the statistical probability (odds ratio) for their incidence in Estonian *P. infestans* populations in 2001–2007

Origin of P. infestans isolates	Virulence factor 2 %	Odds ratio vs. control	p-value	Virulence factor 6 %	Odds ratio vs. control	p-value	Virulence factor 8 %	Odds ratio vs. control	p-value	Sample size N
Phytosanitary quality of seed potato										
High quality[a]	45	0.20	<0.0001	39	0.30	0.0012	24	0.23	<0.0001	186
Low quality[b]	67	Control	-	51	Control	-	57	Control	–	180
Phase of epidemic[c]										
Beginning	54	1.43	0.1391	42	0.87	0.5670	35	2.07	0.0041	180
Advanced phase	41	Control		42	Control		21	Control		156
Total population	49			42			31			366

[a] High quality seed was propagated in the open field 2 years after meristem phase

[b] Low quality seed is propagated from commercial certified seed multiplied in the open field for several consecutive years

[c] Tuber isolates were grouped with isolates in the beginning and middle season with end of season isolates

There were 17 virulence pathotypes among 186 isolates collected from plants with high seed quality while 69 different pathotypes were determined among 180 isolates collected from potato with low seed quality (Table 6). Two pathotypes (1.3.4.7.10.11 and 1.2.3.4.7.10.11) comprised 59 % and 22 % of the population collected from plants propagated with high-quality seed and there were 8 unique pathotypes. In the population collected from plants with low seed quality none of the single pathotypes constituted more than 10 % of the population and 42 unique pathotypes were present.

The overall normalized Shannon diversity index was 0.50. The diversity index was much higher in isolates from low-quality seed potatoes, 0.74 ($F12$=15.138; p=0.0021) (Fig. 1), than in those from high-quality seed potatoes, 0.46.

The pattern of response to the fungicide metalaxyl was very similar among isolates collected either from plants produced from high- or low-quality seed (Table 7).

Phase of Epidemic

The ratio of A1/A2 mating type in the *P. infestans* population during the development of the epidemics shifted rapidly from a 1/1 balance into A1 dominance (68 %) towards the end of the epidemic. At the beginning of the epidemic, the probability for A2 incidence was approximately 2.5 times higher (odds ratio beginning vs. end 2.54; p=0.0016) than at the end of the epidemic (Table 2).

The phase of the epidemic had little effect on the occurrence of universal virulence factors in the population (Table 3). Epidemic phase did not affect on the probability of the occurrence of virulence factors 2 or 6 but virulence factor 8 was more common at the beginning of epidemic than at the advanced phase (odds ratio 2.07, p 0.0041) (Table 4). The probability for the occurrence of virulence factor 5 was 2.2 times (Odds ratio 2.16; p=0.0755) and occurrence of virulence factor 9 was 2.4 times (Odds ratio

Table 5 Effect of the phytosanitary quality of seed potato and the phase of potato late blight epidemic to the prevalence (%) of relatively rare virulence factors 5 and 9 and the statistical probability (odds ratio) for their incidence in Estonian *P. infestans* populations in 2001–2007

Origin of *P. infestans* isolates	Virulence factor 5 %	Odds ratio vs. control	p-value	Virulence factor 9 %	Odds ratio vs. control	p-value	Sample size N
Phytosanitary quality of seed potato							
High quality[a]	7	0.21	0.0493	10	0.19	0.0340	186
Low quality[b]	13	Control	-	15	Control	–	180
Phase of epidemic[c]							
Beginning	11	2.16	0.0775	14	2.44	0.0220	180
Advanced phase	5	Control		6	Control		156
Total population	9			11			366

[a] High quality seed was propagated in the open field 2 years after meristem phase

[b] Low quality seed is propagated from commercial certified seed multiplied in the open field for several consecutive years

[c] Tuber isolates were grouped with isolates in the beginning and middle season with end of season isolates

Table 6 Effect of phytosanitary quality of seed potato on the incidence of different virulence pathotypes of *P. infestans* in Estonia in 2001–2007

Phytosanitary quality of seed	Virulence pathotype	Number of pathotypes	Number of isolates	Percentage of population
High quality[a]	1.3.4.7.10.11	1	109	59
	1.2.3.4.6.7.10.11	1	41	22
	1.3.4.7.8.10.11	1	8	4
	1.2.3.4.7.10.11	1	6	3
	1.2.3.4.6.7.8.10.11	1	5	3
	Other different pathotypes	12	17	9
Low quality[b]	1.2.3.4.7.8.10.11	1	18	10
	1.2.3.4.6.7.10.11	1	15	8
	1.2.3.4.6.7.8.10.11	1	14	8
	1.2.3.4.7.10.11	1	12	7
	1.3.4.7.10.11	1	10	6
	1.2.3.4.6.7.8.9.10.11	1	7	4
	1.2.3.4.5.6.7.10.11	1	7	4
	1.3.4.7.8.10.11	1	7	4
	Other different pathotypes	66	90	50

[a]High quality seed was propagated in the open field 2 years after meristem phase

[b]Low quality seed is propagated from commercial certified seed multiplied in the open field for several consecutive years

2.44; p=0.0220) higher at the beginning than at the advanced phase of the epidemic (Table 5).

The normalized Shannon diversity index did not show statistical differences between isolates collected from different phases of the epidemic (*F13*=0.252; *p*=0.781) (Fig. 2). Nevertheless, at the beginning of the epidemic, the Shannon diversity index was slightly higher (0.55) than at later stages (0.47).

The proportion of metalaxyl-resistant and tolerant isolates was high at the beginning of the epidemic, 39 % and 40 %, respectively. At the end of the epidemic, the probability for the incidence of metalaxyl-sensitive isolates had increased fourfold (odds ratio for sensitive isolates 0.27; p=0.0008) compared to the early phase of the epidemic (Table 7).

Discussion

The average proportion of the A2 mating type in the current study was 39 %, similar to that found in previous studies in Estonia (Runno-Paurson et al. 2009; Runno-Paurson et al. 2010a, b). The overall mating type ratio in Estonia in the 2000s seems to be closer to that reported from the Nordic Countries (Lehtinen et al. 2008) than the ratio reported from

Table 7 Effect of the phytosanitary quality of seed potato and the phase of potato late blight epidemic to the prevalence (%) and statistical probability (odds ratio) for the incidence of metalaxyl sensitive, tolerant and resistant isolates in Estonian *P. infestans* populations in 2001–2007

Parameter	Response to metalaxyl						
	Sensitive isolates %	Odds ratio vs. control	Tolerant isolates %	Odds ratio vs. control	Resistant isolates %	Odds ratio vs. control	Sample size N
Phytosanitary quality of seed potato							
High quality[a]	30	1.49 *p*=0.6775	33	0.72 *p*=0.3073	37	1.22 *p*=0.5249	108
Low quality[b]	27	Control	41	Control	32	Control	71
Phase of the epidemics							
Beginning	21	0.27 *p*=0.0008	40	1.67 *p*=0.1965	39	2.30 *p*=0.0469	108
2 weeks later	26	0.36 p=0.0883	42	2.01 p=0.2259	32	1.52 p=0.4975	19
Advanced	50	Control	27	Control	23	Control	44
Total population	29		36		35		179

[a] High quality seed was propagated in open field 2 years after meristem phase

[b] Low quality seed is propagated from commercial certified seed multiplied in the open field for several consecutive years

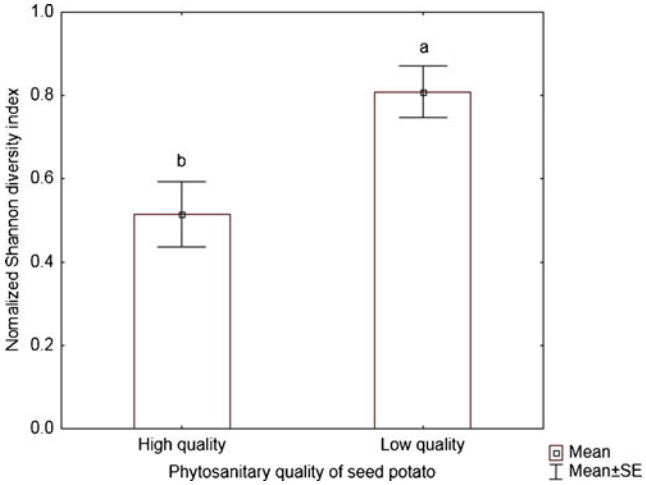

Fig. 1 Normalized Shannon diversity index values of *Phytophthora infestans* isolates collected from different phytosanitary quality of seed potato. Values with different letters differ significantly (Turkey HSD test, $p<0.05$) from each other

the rest of Europe, where the incidence of the A2 mating type has increased only very recently (Cooke et al. 2011).

In the current study, the frequency of virulence factors is similar to that described recently in Estonia (Runno-Paurson et al. 2009; Runno-Paurson et al. 2010a; Runno-Paurson et al. 2010b), in Denmark and Sweden (Lehtinen et al. 2008) and in Germany (Bouws and Finckh 2007), except for virulence factor 9. The prevalence of virulence factor 9 was close to that reported from Polish late blight populations in 2003 and 2004 (Śliwka et al. 2006).

The mean number of virulence factors per isolate of 7.1 is the highest value found for Estonian *P. infestans* populations compared with previous population studies in 2002–2003 (6.3, Runno-Paurson et al. 2009) and in 2004-2007 (6.6, Runno-Paurson et al. 2010a). In 2001 and 2005, the mean

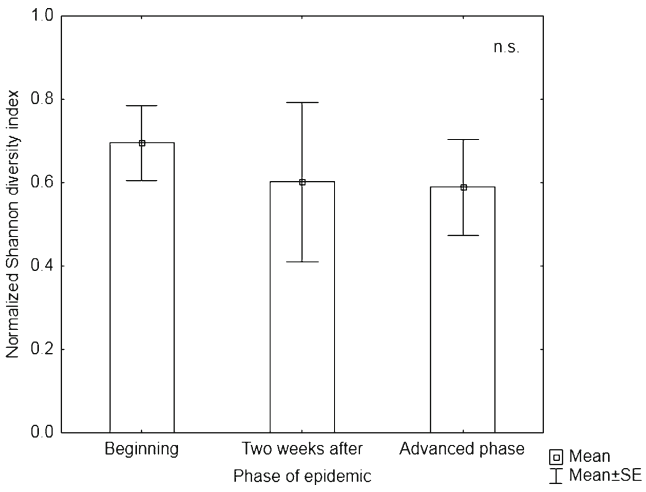

Fig. 2 Normalized Shannon diversity index values of *Phytophthora infestans* isolates collected from different phases of an epidemic

number of virulence factors per isolate was very high (7.8 and 7.4, respectively), greatly influencing the overall results.

Even though metalaxyl-based fungicides were not used in the fields where isolates for this study were collected, metalaxyl-tolerant and resistant strains were frequently present. These results concur with Möller et al. (2009) that this is due to a high rate of migration of resistant isolates from surrounding infected potato fields into unsprayed fields. Metalaxylbased fungicides are widely used in Estonian potato production to control late blight, especially in years of severe late blight risk (2002, 2004 and 2005). This explains why occurrence of metalaxyl resistance in *P. infestans* populations fluctuates from year to year and is strongly connected with high potato late blight incidence and resulting intensive use of metalaxyl-based fungicides.

Phytosanitary Quality of Seed and Phenotypic Traits of the Late Blight Population

Phytosanitary seed quality had no effect on mating type ratio in these populations. This concurs with the study by Bouws and Finckh (2007). Also, the response to metalaxyl in populations was independent of the phytosanitary quality of seed potatoes.

There were differences in racial structure between quality groups of seed potatoes. The two prevalent races 1.3.4.7.10.11 and 1.2.3.4.6.7.10.11 among isolates from high-quality seed potatoes and also overall, were not the most common in the low-quality group (Table 6). Both races have also been common in recent years in Nordic (Lehtinen et al. 2008) and Russian populations (Vedenyapina et al. 2002; Zoteyeva and Patrikeeva 2008). Day et al. 2004 noted that with increased sampling, rarer phenotypes will be found explaining the higher diversity values in Estonian and West European varieties compared to other varieties.

Race diversity, calculated by the normalized Shannon diversity index, showed similar values to those from Estonia for 2004–2007 (0.54, Runno-Paurson et al. 2010a). The higher value of the diversity index on isolates from low-quality seed potatoes (0.74) compared to other isolates shows that more frequent renewal of seed tubers with micro-propagated multiplication methods keeps potato seed material healthy.

The certified lower quality seed tubers have probably been multiplied in several different regions and therefore have accumulated isolates from several different sub populations of *P. infestans* containing numerous different virulence races. The high quality "meristem originated" material has been exposed only to the *P. infestans* sub population close to EVIKAs trials containing a more limited number of virulence races.

Also the time of multiplication in the open field must have an effect on population structure. Within two years in

the open field the potato must have been exposed to a lower number of virulence races than those materials exposed to attacks of *P. infestans* for numerous years in the open field. The yearly and regional variation in mating type ratio and metalaxyl resistance is less than that in virulence races and is therefore not reflected that much in generations seed when grown in the open field.

The isolates collected at the latter part of the epidemic were included in the comparisons of effects of phytosanitary quality of seed potato to keep the statistical model balanced though it is obvious that the effect of seed quality is less evident at late than at early phase of the epidemic. The major impact of the epidemic phase was the decrease of proportion of A2 mating type and metalaxyl tolerant and resistant isolates during the epidemic and the change was similar in *P. infestans* populations obtained from plants produced with high and low quality seed.

Phase of Epidemic

The proportion of A2 and A1 mating types was almost equal (47/53) among isolates collected at the beginning of disease outbreak while later on the A1 mating type became dominant. This finding may indicate an accumulation of infection source (infected mother tubers and infection from allotments) and aggressiveness of the pathogen at the beginning of the epidemic. A more plausible explanation for this finding is possible soil contamination with oospores that survived. Although overwintering of oospores and soilborne infections has not been studied in Estonia, there is strong evidence for it from the consistently high proportion of A2 mating type and both mating types together in the same field (Runno-Paurson et al. 2010a, b). Oospores overwinter in the soil in the Nordic countries (Andersson et al. 1998; Lehtinen and Hannukkala 2004). Lehtinen et al. (2007) showed that the proportion of A1/A2 mating types is close to 1/1 at the beginning of an oospore-derived epidemic and that, during the course of the epidemic, one mating type may become dominant. Isolates collected at different epidemic phases were studied by Runno-Paurson et al. (2010a), but the results did not indicate any changes in mating type ratio during the epidemic progress. In the current study the shift towards one dominating mating type was very clear.

The phase of epidemic progress at the time of sampling *P. infestans* isolates during the seven-year period had a remarkable effect on frequency of metalaxyl resistant and tolerant individuals in the population. The proportion of resistant isolates was highest at the beginning of the late blight epidemic (39 %). By contrast, Dowley et al. (2002) reported that during an 18–year period, resistance was always lower at the beginning of the season and increased as the season progressed. They also stated that phenylamide-resistant strains of *P. infestans* do not overwinter in tubers as effectively as sensitive strains. These studies suggest that plants became infected from both sources – from infected tubers and also from diseased potato plants in neighboring allotments. Private allotment gardens in which potatoes are grown are very common in Eastern Europe. Due of low quality and old potato seeds, these gardens are the main sources of infection for potato production. Möller et al. 2009 tested early and late samples and did not find differences in the percentage of resistant late blight isolates.

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