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Characterisation of *Phytophthora infestans* isolates collected from potato in Estonia during 2002–2003

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Abstract A collection of 101 isolates of *Phytophthora infestans*, obtained from seven sampling sites representing central, east and south-east Estonia during 2002 and 2003 were assessed for several phenotypic and genotypic markers. All 101 isolates were assessed for virulence and resistance to metalaxyl. Virulence to each of the 11 classic resistance genes was found among the tested isolates. The mean number of virulences per isolate was 6.3, with a very low frequency of virulence against resistance genes R5 (5%) and R9 (14%). The most common pathotypes were 1.3.4.7.8.10.11 and 1.3.4.7.10.11, representing altogether 12% of the studied strains. In terms of metalaxyl resistance, 30 resistant, 52 intermediate and 19 sensitive isolates were found. A subgroup of 50 isolates was assessed for mating type, allozymes [glucose-6-phosphate isomerase (Gpi) and peptidase (Pep)], DNA fingerprints with probe RG57 and mtDNA haplotype. Of this subset, 30 were A1 and 20 were A2. Collections from three of the seven fields contained both mating types. Allozyme analysis did not reveal any polymorphism. However, 19 diverse RG57 fingerprints were detected, and two mitochondrial DNA haplotypes, Ia and IIa, were detected. By combining the mating type, mtDNA haplotype and RG57 fingerprint data, 26 multilocus genotypes were identified, of which 18 were detected only once. Genotypic diversity measured by the normalised Shannon diversity index was high (0.76). The large number of multilocus genotypes and the presence of both mating types in some fields indicate that sexual reproduction may take place in Estonian populations of *P. infestans*.

Keywords Diversity · Mating type · Metalaxyl resistance · mtDNA · RG57 fingerprinting · Virulence phenotype

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Introduction

Phytophthora infestans, the causal agent of late blight disease of potato and tomato, is one of the most damaging microbial pathogens world-wide. It has been reported repeatedly that the population structure of *P. infestans* has undergone major changes in

Europe during the past 30 years (Fry et al. 1993; Gisi and Cohen 1996). The predominant 'old' population was apparently displaced by a 'new' population, probably introduced into Europe in the late 1970s by a potato shipment from central Mexico (Fry et al. 1993). The old population consisted of only the A1 mating type and Ib mitochondrial (mt) haplotype isolates, whereas the new population was comprised of isolates of both A1 and A2 mating types, and Ia and IIa mtDNA haplotypes (Spielman et al. 1991; Day and Shattock 1997; Lebreton and Andrivon 1998). In some European countries, *P. infestans* apparently reproduces sexually. Recent reports on the sexual reproduction of *P. infestans* have been published from The Netherlands (Drenth et al. 1994; Zwankhuizen et al. 2000), Sweden (Anderson et al. 1998), Norway and Finland (Brurberg et al. 1999; Hannukkala et al. 2007) and Poland (Śliwka et al. 2006). The old population was generally sensitive to phenylamides but the new population contained resistant/tolerant isolates (Goodwin et al. 1998).

Interaction between the opposite mating types induces the formation of antheridia and oogonia, enabling sexual recombination and producing oospores. Oospores can withstand unfavourable conditions and survive in the soil, thus affecting the epidemiology of the disease (Mayton et al. 2000). Additionally, sexual reproduction increases the genotypic variability of the organism, and may result in an increased virulence and/or fungicide resistance/tolerance (Fry et al. 1993).

Except for very limited reports by Goodwin et al. (1994) and Sujkowski et al. (1994) there are no genetic data characterising *P. infestans* populations in Estonia. The main objectives of this study were to learn the general characteristics of the Estonian population of *P. infestans* in terms of pathotypic diversity, neutral marker diversity, and reaction to metalaxyl/mefenoxam, and then to test the hypothesis that the Estonian population is different from other populations in Europe.

Materials and methods

Collection and culture of isolates

During 2002–2003, 101 isolates of *P. infestans* were collected at seven sites (potato fields) from five locations in Estonia (Fig. 1). Three sites were conventional farm fields: Ingliste 2002 (59°01′N, 24°58′E), Ingliste 2003 (59°02′N, 24°57′E) and Kehtna 2003 (58°56′N, 24°49′E) in central Estonia (Table 1). Metalaxyl-based fungicides were used twice at Ingliste 2003 and Kehtna 2003. These conventional farms represented the most important seed and ware potato-growing area in Estonia. Farmers use high-quality certified seed potatoes and make five to seven fungicide applications (depending on the year) to suppress late blight. Two sites were experimental field trial plots at the Jõgeva Plant Breeding Institute in eastern Estonia: Jõgeva 2002 (58°44′N, 26°25′E) and Jõgeva 2003 (58°44′N, 26°26′E). This location is characterised by high genetic diversity of the host plants including several genotypes with race-specific genes, and with no use of late blight fungicides. Samples were also collected from two small-scale farm fields in central Estonia: Käru 2003 (58°48′N, 25°08′E) and south Estonia: Võnnu 2003 (58°17′N, 27°02′E). In these small-scale farms, farmers use seed potatoes of uncertain quality (often infected with diverse viruses) and sometimes do not rotate. Late blight control practices are highly diverse, ranging from no sprays to very frequent sprays. Metalaxyl-based fungicide was used twice at Võnnu 2003.

Isolates were obtained from all sites. Six to forty leaflets, each with single lesions (one per plant), were collected at the beginning of the epidemic. Isolations were carried out by placing a fragment of infected leaf tissue between ethanol and flame-sterilised tuber slices. Tubers of susceptible cultivars without known R genes were used (Berber in 2002; Bintje in 2003). The slices were put into sterile Petri dishes with a moist filter paper disk on top. The Petri dishes were incubated 6–7 days at 16°C in a growth chamber until the mycelia had grown through the slice. A small amount of mycelia from 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 every 2 months. All phenotypic tests were carried out in October-November of the year of isolation. Genotypic analyses were done in May-July 2004. For some analyses (details below) a subset of 50 isolates, including isolates from each geographic region (Table 2), was evaluated.

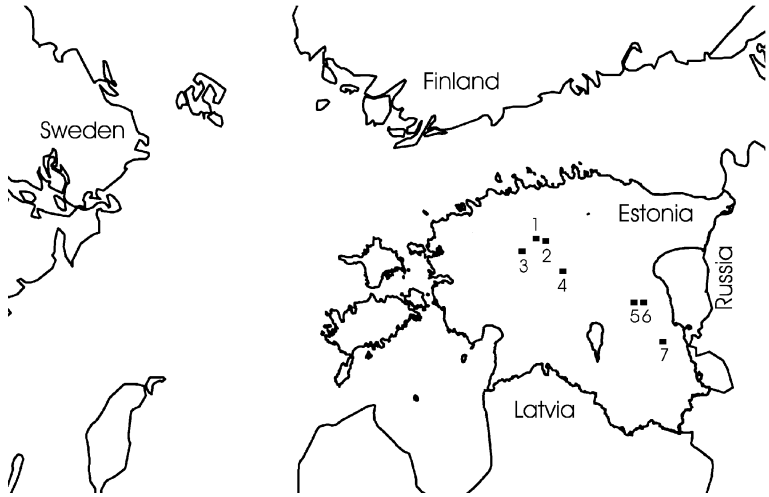

Fig. 1 Map of Estonia showing the sites where the isolates of *Phytophthora infestans* were collected during 2002 and 2003 (see also Table 1). 1 = Ingliste 2002, 2 = Ingliste 2003, 3= Kehtna 2003, 4 = Käru 2003, 5 = Jõgeva 2002, 6 = Jõgeva 2003, 7 = Võnnu 2003

Response to metalaxyl

The resistance to metalaxyl of all isolates was tested using a modification of the floating-leaflet method (Hermansen et al. 2000). Leaflets of susceptible cultivars (Berber in 2002; Bintje in 2003) were obtained from five week-old greenhouse-grown plants. The metalaxyl concentrations were 0.0, 10.0 or 100.0 mg l^{-1} prepared from Analytical Master Standard, CGA 48988 (Ciba Geigy, purity 99.6). The sporangia were multiplied on rye B agar and collected in distilled water with a pallet. 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 water 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 >90% relative humidity (RH). 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 according to 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. Sporulation was regarded as present if the cumulative score for all four leaflets was at least 12. The isolates were rated as resistant if they sporulated on leaflets in 100 mg l^{-1} metalaxyl. Those sporulating on leaflets 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.

Table 1 Sampling of *Phytophthora infestans* isolates collected from Estonia (2002–2003)

Region	Sites[a]	No of isolates characterised for metalaxyl resistance and virulence	No of isolates characterised for molecular and biochemical markers
Central	Ingliste 2002 (1)	6	2
	Ingliste 2003 (2)	10	6
	Kehtna 2003 (3)	12	4
	Käru 2003 (4)	6	5
Eastern	Jõgeva 2002 (5)	41	13
	Jõgeva 2003 (6)	14	9
Southern	Võnnu 2003 (7)	12	11
Total	7	101	50

[a] Numbers in parentheses indicate the site number as in Fig. 1

Virulence tests

The specific virulence of each of the 101 isolates was determined by using Black's differential set of potato genotypes containing resistance genes R1-R11 (Malcolmson and Black 1966) (provided by the

Table 2 Summary of molecular and phenotypic analyses of Estonian isolates of *Phytophthora infestans*, 2002–2003

Multilocus genotype	RG57 fingerprint[a]	RG57 genotype[b]	Mating type	mtDNA haplotype	Metalaxyl resistance[c]	Number of isolates	Number of site	Site name
EE-1	10101010010011010000110011	I	A1	Ia	I	3	2	Jõgeva 02–03
EE-2	10101010010011010000110011	I	A1	IIa	I, R,S	7	2	Ingliste 02–03
EE-3	10101010010011010000110011	I	A2	Ia	I	3	2	Kehtna 03, Võnnu 03
EE-4	10101010010011010110110011	II	A1	Ia	I, R	5	3	Jõgeva 02–03, Võnnu 03
EE-5	10101010010011010110110011	II	A1	IIa	I	1	1	Võnnu 2003
EE-6	10101010010011010110110011	II	A2	IIa	R, S	2	1	Võnnu 2003
EE-7	11100110010011000000110011	III	A1	IIa	S, I, R	6	1	Jõgeva 2002
EE-8	00101010010011000000110011	IV	A2	Ia	I, R	4	1	Käru 2003
EE-9	10001000010011010000110011	V	A2	Ia	R	1	1	Jõgeva 2003
EE-10	10001000010011010000110011	V	A2	IIa	I	1	1	Jõgeva 2003
EE-11	11100011010011010000110011	VI	A2	IIa	I	2	1	Võnnu 2003
EE-12	11101010010011010110110011	VII	A2	Ia	I	1	1	Võnnu 2003
EE-13	11101010010011010110110011	VII	A2	IIa	I	1	1	Võnnu 2003
EE-14	11101110010011010000110011	VIII	A1	IIa	S	1	1	Võnnu 2003
EE-15	11101110010011010000110011	VIII	A2	IIa	I	1	1	Võnnu 2003
EE-16	10100010010011000000110011	IX	A2	Ia	I	1	1	Jõgeva 2003
EE-17	10100110000011000000110011	X	A1	IIa	R	1	1	Ingliste 2002
EE-18	10100110010011000000110011	XI	A1	IIa	I	1	1	Kehtna 2003
EE-19	10101000000011010110110011	XII	A1	Ia	R	1	1	Jõgeva 2002
EE-20	10101010010011000000110011	XIII	A1	IIa	S	1	1	Jõgeva 2002
EE-21	10101011010011000000010011	XIV	A1	Ia	I	1	1	Jõgeva 2003
EE-22	10101011010011010000110011	XV	A2	Ia	I	1	1	Kehtna 2003
EE-23	10101011010011010110110011	XVI	A1	IIa	R	1	1	Jõgeva 2003
EE-24	11100010010011010000110011	XVII	A2	Ia	R	1	1	Käru 2003
EE-25	11101010010011000000110011	XVIII	A1	IIa	R	1	1	Jõgeva 2002
EE-26	10001001010011010110110011	XIX	A2	Ia	R	1	1	Jõgeva 2003

[a] RG57 fingerprint is denoted using ′1′ and ′0′ to indicate presence or absence, respectively, of bands 1–25 recognised by the RG57 probe (Goodwin et al. 1992). [b] RG57 genotypes are numbered with Roman figures consecutively according to Bakonyi et al. (2002b). [c] S, sensitive; I, intermediate; R, resistant.

Scottish Agricultural Science Agency, UK). 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 ($1.0–4.0\times10^4$ 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 RH and were incubated at 16˚C with a 16-h light period, and 8-h dark period. The interactions between the pathogen and potato genotypes were scored seven days after inoculation, using the same scale as indicated for the assessment of metalaxyl resistance. The reaction was compatible if sporulation was detected at least in four leaflets out of six, and the cumulative score was at least 15. Compatible interactions were usually indicated by large, sporulating

lesions. The mean number of virulences per isolate and pathotype were calculated using formulae described by Andrivon (1994).

Mating type determination

Mating type was determined for the subset of 50 isolates (Table 1 and Table 2). Determinations were conducted at Cornell University by growing each sample isolate together with the appropriate tester strain (US970001 for the A1 mating type and US940480 for the A2 mating type) in a Petri dish containing rye agar. Plates were scored for oospore formation at the hyphal interface between the developing colonies after growth for 10–18 days at 16°C in darkness. Isolates forming oospores on plates with the A1 mating type but not the A2 were registered as A2; isolates that formed oospores with the A2 mating type but not the A1 were registered as A1.

Neutral marker assessments

The subset of 50 isolates was tested for neutral markers (allozyme genotype and RFLP fingerprint). Genotypes at the Glucose-6-phosphate isomerase (Gpi) and Peptidase (Pep) loci were assessed using cellulose acetate electrophoresis (Helena Laboratories, Beaumont, TX, USA) following protocols published earlier (Goodwin et al. 1995). Mycelia were obtained from 7- to 10 day-old colonies grown on rye B agar. Isolates of the US-1 and US-8 clonal lineages were used for comparison. These two isolates had Gpi genotypes of 86/100 and 100/111/122, respectively, and Pep genotypes of 92/100, and 100/100, respectively. RFLP analysis was performed using the RG57 probe (Goodwin et al. 1992). This probe recognises a dispersed, moderately repetitive and highly polymorphic DNA element that allows the characterisation of up to 30 bands in a single hybridisation experiment (Fry et al. 1992; Goodwin et al. 1992). Extraction of genomic DNA was done according to the protocol described by Goodwin et al. (1992). The pathogen was grown for 15 days at 18°C in pea broth supplemented with CaCO3, in still culture. DNA was digested with the restriction endonuclease EcoRI, subjected to 0.8% agarose gel electrophoresis and transferred to a nylon membrane (Amersham, Buckinghamshire, UK) as described by Goodwin et al. (1992). Labelling of the PCR-amplified probe was performed using the Random Primers DNA Labelling System kit (Invitrogen, Carlsbad, CA) with P32 labelled dATP. For detection, the membranes were washed once in 2X SSC, 0.1 % SDS for 5 min followed by 10 min washes in 1X SSC, 0.1% SDS and then in 0.1X SSC, 0.1% SDS, respectively (all washes at 65°C), followed by autoradiography (Goodwin et al. 1992). The DNA fingerprinting of the Estonian isolates was determined by comparing their patterns with those of three reference isolates (US-1, US-8 and US-17 clonal lineages).

The mitochondrial DNA (mtDNA) haplotype of each of the 50 isolates in the subset was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), a variation of the method described by Griffith and Shaw (1998). Primer pairs 1 (F1- GCAATGGGTAAATCGGCTCAA; R1- AAACCATAAGGACCACACAT) and 2 (F2—TTCCCTTTTGTCCTCTACCGAT; R2—GCTTATGCTTCAGTTGCTCAT) designed by Griffith and Shaw (1998) were used to amplify mtDNA regions by PCR. Each PCR reaction (50 µl) consisted of 10 X buffer (5 µl), 2.75 µl of $MgCl_2$ (50 mM), 5 µl of dNTPs (2 mM), 1.63 µl of each primer, 32 µl of dH2O, 0.5 µl of Taq and 1.5 µl of genomic DNA. Amplification was conducted with one cycle of 90°C for 90 s followed by 40 cycles of 90°C for 30 s, 55°C for 30 s, and 72°C for 90 s. Products were numbered according to the primer pairs used for amplification. Products P1 and P2 were digested with restriction enzymes *Hha*I (Invitrogen) and *Msp*I (New England Biolabs), respectively. Haplotypes Ia, Ib, IIa and IIb were identified by their restriction patterns as described by Griffith and Shaw (1998).

Data analysis

Each isolate was characterised by a multilocus genotype consisting of DNA fingerprint bands, mtDNA haplotype and mating type. Genotypic diversity as well as race diversity was calculated with the normalised Shannon diversity index (Sheldon 1969): $Hs = -\Sigma g_i \ln g_i / \ln N$, where g_i is the frequency of the *i*th multilocus genotype and N the sample size. The normalised index ranges from 0 (no diversity) to 1 (each isolate represents a unique genotype). Polymorphic bands of the RG57 fingerprints were scored as present (1) or absent (0).

Statistical analyses were performed with 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 logistic analysis (GENMOD procedure in SAS), with the probability of detecting type A2 as the response variable. Analogous logistic analyses were used to examine the differences in the resistance to metalaxyl (a multinomial response variable) between sites and years, and also between the mating types. To determine the differences between years, only two locations (Jõgeva and Ingliste) were included because other locations were sampled only in one year. To determine if there were differences among the different populations, or among different potato R genes, in terms of specific viruluence, Type III ANOVA and Tukey HSD post-hoc tests (α=0.05) were applied.

Results

One-hundred and one isolates of *P. infestans* were collected during 2002–2003 from seven populations in Estonia. All of these isolates were characterised for metalaxyl resistance and for specific virulence.

Metalaxyl resistance

Among the 101 isolates, 30 were resistant, 52 intermediate and 19 sensitive to metalaxyl (Table 2). In 2002, the percentage of resistant isolates was 36.2% and in 2003 it was 24.1%, but the difference was not significant (chi-square = 2.43, df=1, P= 0.1192). All sites had isolates with some insensitivity (intermediate and/or resistant), with the samples from Ingliste 2003 and Käru 2003 containing only resistant and intermediate isolates (Table 3). In 2003, there were no significant differences among sites (chi-square = 1.31, df=4, P=0.86).

Virulence

There was virulence to each of the 11 R genes in the sampled population. Isolates with virulence to differentials with R1, R3, R4, R7, R10 and R11 were common, but virulence to R5 (5%±2.4 SE) and to R9 (14%±7.2 SE) were somewhat rare ($F_{(10,66)}$=15.89, P<0.0001) (Fig. 2). Most of the isolates (>90%) were able to overcome four or more R genes, but there were high levels of diversity with 66 pathotypes detected (Table 4). The mean number of virulences per isolate was 6.3 and ranged among sites from 4.3 to 7.3 (Table 5). The normalised Shannon diversity index was 0.92. The most common pathotypes 1.3.4.7.8.10.11 and 1.3.4.7.10.11 made up only 12% of the isolates tested and nearly half of all pathotypes (>48%) were detected only once. There were significant differences in terms of specific virulence among the five populations ($F_{4,10}$=3.79, P=0.017). However, there were no differences between the two years ($F_{(1,10)}$=8.30, P=0.06) (Table 4).

Mating type

Within the subset of 50 isolates, 30 were A1 and 20 were A2 (Table 2). A1 mating type individuals were detected in six of the seven samples, and A2 mating type individuals were identified in four samples (Table 2). There were three samples that contained both A1 and A2 mating type individuals (Table 2). However, among the five sites in 2003 there were significant differences (chi-square=18.54, df=4, P< 0.001) in the proportions of A1 and A2.

Metalaxyl-resistant strains occurred in both mating types (Table 2). The 30 A1 isolates tested included nine resistant, 15 intermediate, and six sensitive. Among the 20 A2 mating type isolates, five were resistant, 14 were intermediate, and one was sensitive to metalaxyl. The level of metalaxyl resistance did not depend significantly on the mating type (chi-square=2.08, df=1, P=0.15).

RG57 analysis

In the subset of 50 isolates, nine bands were not detected (bands 4, 9, 11, 12, 15, 17, 19, 22, 23) and five occurred in all isolates (bands 13, 14, 21, 24 and 25). The remaining 12 bands were polymorphic (bands 1, 2, 3, 5, 6, 7, 8, 10, 16, 18, 20). A total of 19 RG57 fingerprints were detected, but four genotypes (I, II, III, and IV) (Table 2) accounted for more than half of the isolates (62%).

Allozyme genotype and mtDNA haplotype

The Gpi and Pep loci were monomorphic in the sampled population. For both glucose-6-phosphate

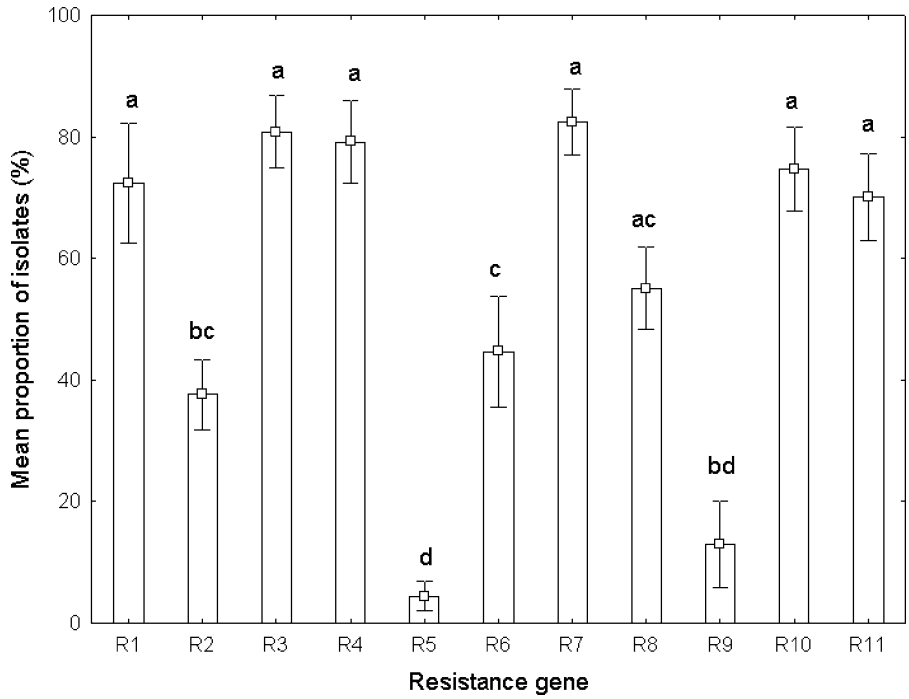

Fig. 2 Frequency (%) of virulence to potato R genes in Estonian populations of *Phytophthora infestans*, 2002–2003. Different letters on the bars indicate significant differences at $\alpha=0.05$ (Tukey HSD test)

isomerase and peptidase, only the *100/100* genotype was detected.

Two mitochondrial haplotypes were detected among the 50 isolates tested; 23 were haplotype Ia and 27 were IIa. These haplotypes occurred in both A1 and A2 individuals (Table 2).

Table 3 Metalaxyl resistance among isolates of *Phythophthora infestans* from different years and locations in Estonia

Site	Metalaxyl resistance[a]			
	S (%)	I (%)	R (%)	Total
Ingliste 2002	33.3	16.7	50.0	6
Ingliste 2003	0.0	60.0	40.0	10
Jõgeva 2002	29.3	36.6	34.1	41
Jõgeva 2003	7.1	64.3	28.6	14
Kehtna 2003	16.7	66.7	16.7	12
Käru 2003	0.0	66.7	33.3	6
Võnnu 2003	16.7	75.0	8.3	12
Total	18.8	51.5	29.7	101

[a] S, metalaxyl-sensitive; I, intermediate metalaxyl-sensitive; R, metalaxyl-resistant.

Multilocus genotypes

Twenty-six multilocus genotypes were identified from the neutral markers. Of these, 18 were detected only once, and most of these were collected from Jõgeva (either 2002 or 2003) (Table 2). Genotypic diversity measured by the normalised Shannon index was 0.76. Three genotypes were common in the Estonian samples: EE-2 was found seven times; EE-7 was found six times; and EE-4 was found five times. Only three multilocus genotypes (EE-1, EE-2 and EE-4) were detected in both years.

Discussion

This study confirms earlier reports (Goodwin et al. 1994; Vorobyeva et al. 1991) that the population of *P. infestans* in Estonia in 2002–2003 shares many of the characteristics typical of populations in other parts of Europe. Since the early 1980s, dramatic changes have been reported repeatedly in the European populations of *P. infestans* with the occurrence of high levels of pathotypic diversity, A2 as well as A1 mating types and the widespread occurrence of metalaxyl resistance.

Table 4 Race (pathotype) frequencies among isolates of *Phytophthora infestans* from Estonia (2002–2003)

Race	Number of virulence factors	Number of isolates
1.3.4.7.8.10.11	7	6
1.3.4.7.10.11	6	6
1.2.3.4.7.8.10.11	8	5
1.2.3.4.6.7.8.9.10.11	10	3
1.2.3.4.6.7.8.10.11	9	3
1.2.3.4.6.7.10.11	8	3
1.2.3.6.7.8.10.11	8	3
1.3.4.6.7.8.10.11	8	3
1.3.4.6.7.10	6	3
1.4.7.8.10.11	7	3
1.2.3.4.6.7.8	7	2
1.3.4.6.7.8.10	7	2
1.2.3.4.6.7	6	2
2.3.4.6.7.10	6	2
3.4.8.10.11	5	2
1.3.4.7	4	2
1.3.7.10	4	2
1.2.3.4.5.6.7.8.10.11	10	1
1.2.3.4.5.6.7.10.11	9	1
1.2.3.4.5.6.7.9.10	9	1
1.2.3.4.6.7.8.9.10	9	1
1.2.3.4.6.7.8.10	8	1
1.2.3.4.6.8.9.10	8	1
1.2.3.4.7.9.10.11	8	1
1.3.4.5.7.8.10.11	8	1
1.3.4.6.7.8.9.10	8	1
1.3.4.6.7.9.10.11	8	1
1.2.3.4.6.7.9	7	1
1.2.3.4.7.10.11	7	1
1.2.3.4.7.9.11	7	1
1.3.4.6.7.10.11	7	1
1.3.4.6.7.8.9	7	1
1.3.4.8.9.10.11	7	1
1.4.6.7.8.10.11	7	1
2.3.4.6.8.10.11	7	1
1.2.3.4.8.10	6	1
1.3.4.7.8.10	6	1
1.3.7.8.10.11	6	1
1.4.6.7.10.11	6	1
3.4.7.8.10.11	6	1
4.6.7.8.10.11	6	1
1.3.4.7.11	5	1
1.3.4.7.8	5	1
1.8.9.10.11	5	1
2.3.4.6.11	5	1
2.4.6.10.11	5	1
3.4.7.10.11	5	1
3.5.7.8.10	5	1
4.7.8.10.11	5	1
1.3.7.11	4	1
1.4.6.7	4	1
1.8.10.11	4	1
2.3.6.10	4	1
2.3.6.7	4	1
3.4.10.11	4	1
3.4.6.7	4	1
3.4.7.11	4	1
4.7.10.11	4	1
3.4.6	3	1
1.3.7	3	1
2.3.8	3	1
1.7.8	3	1
3.7.10	3	1
3.7.11	3	1
7.11	2	1
3	1	1
Total number of isolates		101
Total number of races		66

These changes have been chronicled by other workers for other countries in Europe (Spielman et al. 1991; Drenth et al. 1994; Sujkowski et al. 1994; 1996; Brurberg et al. 1999; Bakonyi et al. 2002a; Day et al. 2004;). Our study indicates that the population of *P. infestans* in Estonia is characterised by high levels of pathotypic diversity, the occurrence of A2 as well as A1 mating types and widespread metalaxyl resistance, which confirm the preliminary suggestions from previous studies

We found differences in the A1/A2 ratio between sites, regions and years in Estonia. This is similar to the situation in other European countries. For example, a higher proportion of the A2 mating type has been found in certain years and/or regions in Germany, Poland and The Netherlands (Schöber and Turkensteen, 1992; Sujkowski et al., 1994;

Table 5 Frequencies of specific compatibility (virulence) to potato R genes in isolates of *Phytophthora infestans* from different locations and years in Estonia

Virulence to resistance gene	Sampling sites						
	Ingliste 2002	Ingliste 2003	Jõgeva 2002	Jõgeva 2003	Kehtna 2003	Käru 2003	Võnnu 2003
R1	100	100	93	64	50	67	33
R2	50	50	46	50	17	33	17
R3	100	80	100	86	75	67	58
R4	83	100	83	71	100	50	67
R5	17	0	7	7	0	0	0
R6	83	10	61	50	33	50	25
R7	100	90	98	64	75	83	67
R8	50	80	44	79	58	33	42
R9	50	0	20	21	0	0	0
R10	81	100	71	86	92	67	58
R11	75	90	49	86	92	67	58
Mean number of virulences/ isolate	7.3	7.0	6.7	6.6	5.9	5.2	4.3
Number of isolates tested	6	10	41	14	12	6	12

Zwankhuizen et al., 2000). We found that in Estonia, A2 and A1 isolates often occurred in the same field, suggesting that sexual reproduction is theoretically possible (Turkensteen et al., 2000).

The proportion of metalaxyl-resistant isolates in Estonia during the period 2002–2003 is in the same range as those in other European countries (Gisi and Cohen, 1996; Hermansen et al., 2000; Bakonyi et al., 2002a; Bakonyi et al., 2002b; Day et al., 2004; Nagy et al., 2006; Lehtinen, et al., 2006). While we found metalaxyl resistance to be more frequent among A1 than A2 isolates, the difference between A1 and A2 mating types in regard to metalaxyl resistance was not dramatically large, and our limited samples preclude further speculation. Metalaxyl-based fungicides are recommended for use only twice each season. Perhaps for this reason, there still remain some isolates that are sensitive or intermediately sensitive to metalaxyl.

The racial structure of the Estonian population of *P. infestans* is quite similar to those reported recently from Finland and Norway (Hermansen et al. 2000), France (Lebreton and Andrivon 1998; Knapova and Gisi 2002), Poland (Zimnoch-Guzowska 1999), and Switzerland (Knapova and Gisi 2002). However, the structure in Estonia differs from that reported for populations in Russia (Elansky et al. 2001), Finland (Hermansen et al. 2000; Lehtinen et al. 2006) and Norway (Hermansen et al. 2000), France (Andrivon 1994; Lebreton and Andrivon 1998; Knapova and Gisi 2002), Switzerland (Knapova and Gisi 2002), Poland (Zimnoch-Guzowska 1999) and in The Netherlands (Schöber and Turkensteen 1992). Two of the most common pathotypes for Estonia (Table 2) were also the most common in Poland in 1992, 1994, 1996, 1997 and 1998 (Zimnoch-Guzowska 1999).

The Estonian race structure in 2002–2003 is highly diverse and complex. Most races were unique, appearing only once (Table 2), and the three most common pathotypes (Table 2) comprised only 16.8% of the population. The mean number of virulence genes per isolate in Estonia (6.3) is similar to that found in Norway (5.8), Finland in 1990–1996 (5.3) (Hermansen et al., 2000) and in 1997–2000 (6.0) (Lehtinen, et al. 2006), the Birobijan region in Russia (5.5) (Elansky et al., 2001), The Netherlands (4.7) (Schöber and Turkensteen, 1992), France (4.7) (Lebreton and Andrivon 1998), Poland in 1985–91 (6.4) (Sujkowski et al., 1996), Eastern Germany in 1985 (7.1) (Sujkowski, et al. 1996), and France and Switzerland (7.6) (Knapova and Gisi, 2002). However, the mean number of virulence genes per isolate in Estonia in 2002–2003 was somewhat lower than in various regions in Russia (Sakhalin 10, Ekaterinburg 8.9, Irkutsk 8.4, Vladivastok 8.3, Khabarovsk 8.3 and Moscow region 8.1) (Elansky et al., 2001).

Race diversity calculated by the normalised Shannon diversity index showed higher values in Estonia (0.92) than in Finland and Norway (Hermansen et al., 2000), The Netherlands (Drenth et al., 1994) and Poland (Sujkowski et al., 1996).

Most of the 19 RG57 fingerprints are apparently unique to Estonia. However, three fingerprints (I, IV, V) (Table 5) were identical with fingerprints identified in Russia, Norway, Great Britain and Finland. Estonian fingerprint I was identical with the Russian fingerprint MO-12 (Moscow region) (Elansky et al., 2001), Norwegian fingerprint N-27 (Brurberg et al., 1999) and British fingerprint RF060 (Day et al., 2004). Fingerprint IV was identical with Russian fingerprint MO-14 (isolated from tomato) and fingerprint V with Russian fingerprints SIB-2 and MO-2 (Elansky et al. 2001), Finnish fingerprint F-6 (Brurberg et al. 1999), Norwegian fingerprint N-3 (Brurberg et al. 1999) and British fingerprint RF008 (Day et al. 2004). Additionally, genotypes I and VII were similar to Dutch fingerprints NL-23 and NL-114 (Zwankhuizen et al. 2000) and fingerprint IV was similar to Northern Ireland fingerprint NI-1a (Cooke et al. 2006). However, Sujkowski et al. (1994) reported that isolates collected in 1983 in Estonia had the same allozyme and DNA fingerprint genotypes as the most common Polish genotype PO-4, which was not found in the present study.

When comparing the results of this study with those of other studies, it is clear that the population from Estonia has significant similarity to populations in Russia, Finland and Norway. Although Estonia grows most of its own seed potatoes and imports only 5% of its seed potatoes from The Netherlands, it is clear that there is sufficient gene flow so that the population of *P. infestans* in Estonia is related to populations in neighbouring countries.

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