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PHENOTYPIC AND GENOTYPIC
CHARACTERISATION OF POTATO LATE BLIGHT
PATHOGEN *PHYTOPHTHORA INFESTANS*
IN ESTONIAN POPULATIONS

KARTULI-LEHEMÄDANIKU TEKITAJA
PHYTOPHTHORA INFESTANS
EESTI POPULATSIOONIDE FENOTÜÜBILINE
JA GENOTÜÜBILINE ISELOOMUSTAMINE

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LIST OF ORIGINAL PUBLICATIONS

This thesis is a review of the following papers, which are referred to by Roman numerals in the text. The papers are reproduced by kind permission of the following journals: European Journal of Plant Pathology (I, IV), Journal of Plant Pathology (II) and Agronomy Research (III, V).

- I. **Runno-Paurson, E.**, Fry, W.E., Myers, K.L., Koppel, M., Mänd, M. 2009. Characterization of *Phytophthora infestans* isolates collected from potato in Estonia during 2002–2003. European Journal of Plant Pathology, **124**, 565-575.
- II. **Runno-Paurson, E.**, Fry, W.E., Remmel, T., Mänd, M., Myers, K.L. 2010. Phenotypic and genotypic characterisation of Estonian isolates of *Phytophthora infestans* in 2004–2007. Journal of Plant Pathology, **91** (in press).
- III. **Runno-Paurson, E.**, Remmel, T., Koppel, M., Tähtjärv, T. 2010. Occurrence and distribution mating types A1 and A2 of *Phytophthora infestans* in eastern Estonia. Agronomy Research (accepted for publication).
- IV. **Runno-Paurson, E.**, Remmel, T., Ojarand, A., Mänd, M. 2010. Do crop management practices influence the structure of *Phytophthora infestans* population? European Journal of Plant Pathology (in review).
- V. **Runno, E.**, Koppel, M. 2006. The question of metalaxyl resistance on late blight fungus in Estonia. Agronomy Research, **4**, 341-344.

Table 1. Author's contribution to each article (%)

	I	II	III	IV	V
Idea and design	90	100	100	100	50
Sampling	100	100	100	60	100
Isolates analysing	100	100	100	65	100
Data analysis, statistics	70	50	60	55	100
Writing	80	90	95	90	80

1. INTRODUCTION

Phytophthora infestans (Mont.) de Bary is one of the most serious and economically-important pathogens of potato worldwide, including Estonia. It causes potato late blight, a devastating disease which is an ongoing threat to potato growers in temperate regions.

In terms of production, potato is currently the fifth most important crop worldwide (FAOSTAT 2007) and feeds more than a billion people from Russia to South America. However, its potential yield is decreased by about 25% each year due to pests and diseases. Late blight is the worst of these diseases and has potential to cause total crop loss in a short time (Fry & Goodwin 1997). Potato late blight and the famine it can cause have been widely studied, from their social impact to policies of food production and distribution. It has been the subject of countless scientific papers, government documents and popular literature.

Under favourable conditions, the pathogen not only reduces yield by destroys foliage and prevents the growth of tubers, it also promotes rotting of the tubers before and during storage (Smart & Fry, 2001) and thereby causes a considerable yield loss. In Estonia, it is not possible to achieve high yield with good quality in conventional production without control against the late blight pathogen (Koppel, 1997). Effective control requires vigilance and often numerous applications of fungicide (Cooke *et al.*, 2003). In organic production, where mostly varieties with high resistance are used the yield loss may reach up to 50% (Runno-Paurson *et al.*, personal observations). Protective copper based fungicides, which are used in organic production systems in Europe are prohibited in Estonia.

The fungal-like oomycete *P. infestans*, can reproduce both sexually and asexually. For sexual reproduction, *P. infestans* requires both A1 and A2 mating types to produce gametangia (Fry & Goodwin, 1997). 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). Oospores have been shown to persist in soil for 3-4 years in the Netherlands (Turkensteen *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). 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 & Cohen, 1996). The centre of diversity of this oomycete is located in the highlands of Mexico (Fry & Goodwin, 1997), where both mating types have always been present. The population is especially virulent and remarkably diverse for neutral genetic markers (Fry *et al.*, 1993).

Previous studies have demonstrated that the population in Mexico is highly sub-structured (Fry *et al.*, 1992). At least two different migration events have occurred from Mexico. The first is postulated to have occurred before 1845, after which time *P. infestans* swept through Europe, being most destructive in Ireland and resulting in the death of over one million people due to starvation, and emigration of 1.5 million people to other parts of Europe or North America (Drenth *et al.*, 1994). Until the 1980s, the A2 mating type was restricted to central Mexico and only the A1 mating type was distributed worldwide (Fry *et al.*, 1993). The second migration occurred in the 1970s to 1990s, bringing the A2 mating type out of Mexico and also containing genetically diverse and aggressive strains (Fry *et al.*, 1992; Fry *et al.*, 1993). Analyses of allozyme markers (Tooley *et al.*, 1985; Shattock *et al.*, 1986) and DNA fingerprints (Goodwin *et al.*, 1992a, b; Drenth *et al.*, 1993) of isolates from a number of locations in the world supported the notion that the aforementioned changes resulted from displacement of an 'old' world-wide clonal lineage (US-1) by a new population (Spielman *et al.*, 1991; Fry *et al.*, 1993; Drenth *et al.*, 1994). The 'new genotypes' also proved to be generally more aggressive than the old clonal lineage (Carlisle *et al.*, 2002; Shattock, 2002). In recent years, in most European populations the 'old' population genotypes have not been detected. *P. infestans* reproduces sexually in most European countries (Zwankhuizen *et al.*, 2000; Bagirova & Dyakov, 1998; Schöber-Butin, 1999; Anderson *et al.*, 1998; Brurberg *et al.*, 1999; Lehtinen *et al.*, 2007; Lehtinen *et al.*, 2008; Avenaño Córcoles, 2007; Śliwka *et al.*, 2006). The proportion of A2 mating type isolates collected from commercial potato fields has remained low in the UK, France, Germany, Belgium and Switzerland (Gisi & Cohen, 1996; Bakony *et al.*, 2002a; Day *et al.*, 2004; Cook, *et al.*, 2006), whereas in the Netherlands and Nordic countries it has reached over 50% (Hermansen *et al.*, 2000; Turkensteen *et al.*, 2000; Lehtinen *et al.*, 2007; Lehtinen *et al.*, 2008).

The control of potato late blight is massively dependent on the use of fungicides (Goodwin *et al.*, 1996). However, the strategies of chemical pest control must consider the consequences of the potential spread of resistance in the pathogen. Phenylamide fungicides are a class of systemic compounds including metalaxyl (Ridomil, oxadixyl, benalaxyl and ofurace) that show excellent protective, curative and eradicated anti-fungal activity and exclusively control diseases caused by Peronosporales (Schwinn & Staub, 1987). Resistance to metalaxyl was first recorded in *P. infestans* in Ireland and in the Netherlands in 1980 (Davidse *et al.*, 1981; Dowley & O'Sullivan, 1981). Although the old population was largely sensitive to phenylamides, phenylamide-resistant isolates belonging to the old population have been reported (Goodwin *et al.*, 1996; McLeod *et al.*, 2001). The proportion of metalaxyl-resistant isolates fluctuates from year to year and within season (Gisi & Cohen, 1996). However, in the 1990s, resistance levels remained more or less stable in all European countries (Gisi & Cohen, 1996; Cooke *et al.*, 2003; Day *et al.*, 2004). The proportion of metalaxyl resistant isolates have decreased to 2-13% in the four Nordic countries (Lehtinen *et al.*, 2008) where in Norway and Finland in the early and mid 1990s approximately 60% of the isolates were resistant to metalaxyl (Hermansen *et al.*, 2000).

Before this study there was little knowledge about *P. infestans* genetics in Estonia, even though research was conducted regularly by the researchers of the All-Union Research Institute of Phytopathology. The race composition of the late blight pathogen was determined during in years 1966-89 from isolates collected from Estonia (Koppel, 1996). No studies on virulence or other epidemiological characters were then made during the next ten-year period. However, Vorobyeva *et al.* (1991), Goodwin *et al.* (1994) and Sujkowski *et al.* (1994) reported data (fingerprints with RG57, allozymes and mating type) about two isolates collected from Estonia in 1983. Since the early 1980s, dramatic changes have been reported repeatedly in the European populations of *P. infestans* with the occurrence high levels of pathotypic diversity, A2 as well as A1 mating types and the widespread occurrence of metalaxyl resistance. These changes have been chronicled by workers from other countries in Europe (Spielman *et al.*, 1991; Drenth *et al.*, 1994; Sujkowski *et al.*, 1994; Sujkowski *et al.*, 1996; Brurberg *et al.*, 1999; Bakonyi *et al.*, 2002a; Day *et al.*, 2004;) but information about Estonian populations was scant. As a further complication, farming practices in Estonia have become more diverse in recent decades. The number of organic farms has in-

creased since early 1990s and risen notably since 2002. About 10 percent of all cultivable land is used for potato production. However, the organic farms in Estonia have various backgrounds, for example, many of them do not rotate crops properly (time is too short) and seed potatoes are often not certified. Moreover, any chemical control against late blight is prohibited in organic farms. Therefore, it can be expected that organic farms have a higher risk of late blight epidemics and consequent yield loss than do conventional farms.

2. AIMS OF STUDY

Knowledge of the population studies of *P. infestans* in Estonia were insubstantial during its occupation in the Soviet Union. All studies were done by researchers of the All Union Institute of Plant Pathology in 1970-80; results of these studies were confidential and never published (Koppel *et al.*, 1996). Except for very limited reports by Vorobyeva *et al.* (1991), Goodwin *et al.* (1994) and Sujkowski *et al.* (1994) there are no genetic data characterizing *P. infestans* populations in Estonia. Therefore the aims 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 (I, II, III, IV, V).

We also sought answers to several more specific questions:

- 1) Does the mating type ratio in Estonia suggest an occurrence of sexual reproduction (I, II, III, IV) and are there any indications of oospore-derived epidemics (II)?
- 2) Is the phenotypic and genotypic variation at the same level as in other European countries as indicated by virulence and DNA-fingerprints (I, II, III, IV)?
- 3) What are the impacts of time and region on various population parameters of *P. infestans* (I, II, III)?
- 4) What is the efficacy of metalaxyl based fungicides in control of potato late blight (V)?
- 5) Do organic productions have more diverse and/or more resistant populations than conventional fields (IV)?

3. MATERIALS AND METHODS

3.1. Collection and culture of isolates

3.1.1. Study sites and isolates sampling

For characterisation of *P. infestans* Estonian population (I, IV, V), during two years (2002-2003), 101 isolates of *P. infestans* were collected at seven sites (potato fields) from five locations (Figure 1; I). Three sites were conventional farm fields: Ingliste 2002, Ingliste 2003 and Kehtna 2003 in central Estonia (Table 2). 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 apply fungicide five to seven times each growing season (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 and Jõgeva 2003. This location is characterized by high genetic diversity of the host plants including several genotypes with race-specific genes, and here late blight fungicides were not used. Samples were also collected from two small-scale farm fields in central Estonia: Kärü 2003 and South Estonia Võnnu 2003. 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.

Table 2. Sampling of *Phytophthora infestans* isolates collected from Estonia (2002-2003)

Region	Sites ^a	No of isolates characterized for metalaxyl resistance and virulence	No of isolates characterized for molecular and biochemical markers
Central	Ingliste 2002 (1)	6	2
	Ingliste 2003 (2)	10	6
	Kehtna 2003 (3)	12	4
	Kärü 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 there in Figure 1 (I).

More detailed characterisation of Estonian population of *P. infestans* was undertaken during 2004-2007 (II, IV, V). Potato leaves infected by *P. infestans* were collected from 25 sites (small and large scale conventional producers and untreated experimental field plots) in six regions representing central, northern, eastern, southern, south-western and south-eastern Estonia (Table 3).

Table 3. Origin and characteristics of *Phytophthora infestans* isolates collected from Estonia (2004-2007)

Sites	Tested for				
	Mating type	Metalaxyl resistance	Virulences	mtDNA haplotype	RG57 fingerprints
Aantsla 2004	14	13	15	4	4
Enge 2004	14	13	13	4	4
Enge 2006	13	13	13	0	0
Enge 2007	16	16	16	0	0
Ingliste 2004	22	20	22	4	4
Ingliste 2005	23	23	25	5	5
Ingliste 2007	15	15	19	0	0
Jõgeva 2004	18	18	19	4	4
Jõgeva 2005	17	17	18	5	5
Jõgeva 2006	30	29	30	0	0
Jõgeva 2007	21	21	23	0	0
Jõusa 2006	15	15	15	0	0
Kalsa 2005	19	19	19	3	5
Kalsa 2006	15	14	15	0	0
Kambja 2004	15	15	15	3	3
Laheotsa 2004	17	17	17	4	4
Laheotsa 2005	10	10	10	5	4
Naha 2005	10	9	10	5	5
Naha 2006	16	15	16	0	0
Naha 2007	14	14	16	0	0
Paalimäe 2004	16	16	11	4	4
Võnnu 2004	24	20	24	4	3
Võnnu 2005	21	21	21	5	5
Võnnu 2006	15	15	15	0	0
Võnnu 2007	14	14	15	0	0
Total	424	412	432	59	59

Conventional producers have been divided into two groups. In the small scale conventional farms, farmers used seed potatoes of uncertain quality and did not follow rotation rules. Chemical late blight treatment was applied occasionally and varied from no sprays to 1-4 sprays. In the large

scale conventional productions, farmers use high-quality certified seed potatoes, planted potatoes no more frequently than every 3th year (with some exceptions) and applied fungicide, usually six – seven times per season, but sometimes as many as 11 times/season. In experimental plots at Jõgeva Plant Breeding Institute (four sites) diverse cultivars and breeding lines were used, and the quality of seed potatoes was also diverse.

To test for differences between crop systems, 196 isolates of *P. infestans* were collected from 12 potato fields (4 organic, 4 small scale conventional and 4 large scale conventional productions) during two years (2004 and 2005) in northern Estonia (Table 4). The small and large scale conventional farms used in this study differed in their usage of agrotechnical methods. In particular, in the small scale conventional farms, farmers used seed potatoes of uncertain quality and did not follow crop rotation. Chemical late blight treatment was applied only once per growing season. In the large scale conventional farms, farmers used high-quality certified seed potatoes, adhered to the official crop rotation requirements and made at least 6-7 treatments against potato late blight per season. In organic productions, metalaxyl was not used, as copper-based fungicides are not allowed in organic farms in Estonia.

Table 4. Origin of *Phytophthora infestans* isolates and numbers of tests for characters in isolates from northern Estonia (2004-2005)

Cropping system	Tested for Mating type (n)	Virulence (n)	mtDNA haplotype (n)
Organic	42	54	24
Small scale conventional	61	68	24
Large scale conventional	72	74	18
Total	175	196	66

Nine to twenty-three leaflets, each with single lesion (one per plant) were collected in organic and small scale farms twice in each year: at the beginning of an outbreak and at the end of the growing season (an approximately equal number of isolates was taken early and late in the season). In early stages of the epidemics, approximately 20-25% of leaf area of the infected plants and less than 10% of plants were infected with late blight. In later stages, about 20-40% of leaf area and more than 50% of the plants were infected. In large scale farms, samples were collected at the beginning of an outbreak. The plants were selected by randomising the distance from field edges, and from each plant the blighted leaf was also randomly chosen, excluding those that had several or no lesions.

To detect temporal changes in population parameters, 133 isolates of *Phytophthora infestans* were collected from Estonia during 2001-2007. All sites used in the seven years were experimental field trial plots at the Jõgeva Plant Breeding Institute. The study area is characterized by high genetic diversity of the host plants including several genotypes that have race specific genes due to potato plant breeding. No fungicides were used for late blight control. Most of the isolates originated from leaves and a minority from tubers.

Isolation was carried out from one typical, single lesion per sample (I, II, III, IV, V). Blighted leaves (one per plant) were collected in the period from the emergence of disease (I, II, III, IV, V) until the end of the growing season (III, IV, V). Six to forty leaflets, each with single lesions (one per plant), were collected at the beginning of the epidemic on both collecting periods.

3.1.2. Culture of isolates

All the isolates collected from the various sites were cultured for characterisation (I, II, III, IV, V). Isolations were carried out by placing a fragment of infected leaf tissue between ethanol and flame-sterilized tuber slices (I, II, III, IV, V). Tubers of susceptible cultivars without known R genes were used (Berber in 2002, 2004-2007; Bintje in 2003). The slices were put into sterile Petri dishes with a moist filter paper disk on top. The Petri dishes were incubated for 6-7 days at 16° C in a growth chamber until the mycelia had grown through the slice. A small amount of mycelia from the tuber slices was then transferred with a sterile needle to rye B agar (Caten & Jinks, 1968). The pure cultures were kept at 5° C and transferred to rye B agar after every 2 months. All phenotypic tests were carried out in October-November of the year of isolation (I, II, III, IV, V). Genotypic analyses were done in May-July 2004 (I, III) and in November-December 2005 (II, III).

3.2. Phenotypic markers

3.2.1. Response to metalaxyl

The resistance to metalaxyl of all isolates was tested using a modification of the floating-leaflet method (Hermansen *et al.*, 2000) modified by author (I, II, IV, V). 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 an 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 microlitres 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 7 days in natural daylight at 15°C and >90% 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 7 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.

3.2.2. Virulence tests

The specific virulence of each of the tested isolates was determined by using Black's differential set of potato genotypes containing resistance genes R1-R11 (I, II, IV) (Malcolmson & 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-microliter drop of sporangial suspension (1.0-4.0 x 10⁴ 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 light period, and 8-h dark period. The interactions between the pathogen and potato genotypes were scored 7 days after inoculation, using the same scale as indicated for the assessment of metalaxyl resistance. The reaction was compatible if sporulation was detected on 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 mean number of virulences per isolate and pathotype were calculated using formulae described by Andrivon (1994).

3.2.3. Mating type determination

For mating type determination a pure culture of each isolate was paired with known A1 and A2 testers in Petri dish containing rye B agar (I, II, III, IV). Plates were scored for oospore formation (Figure 1) at the hyphal interface between the developing colonies after growth for 10-18 days at 16°C in the dark.

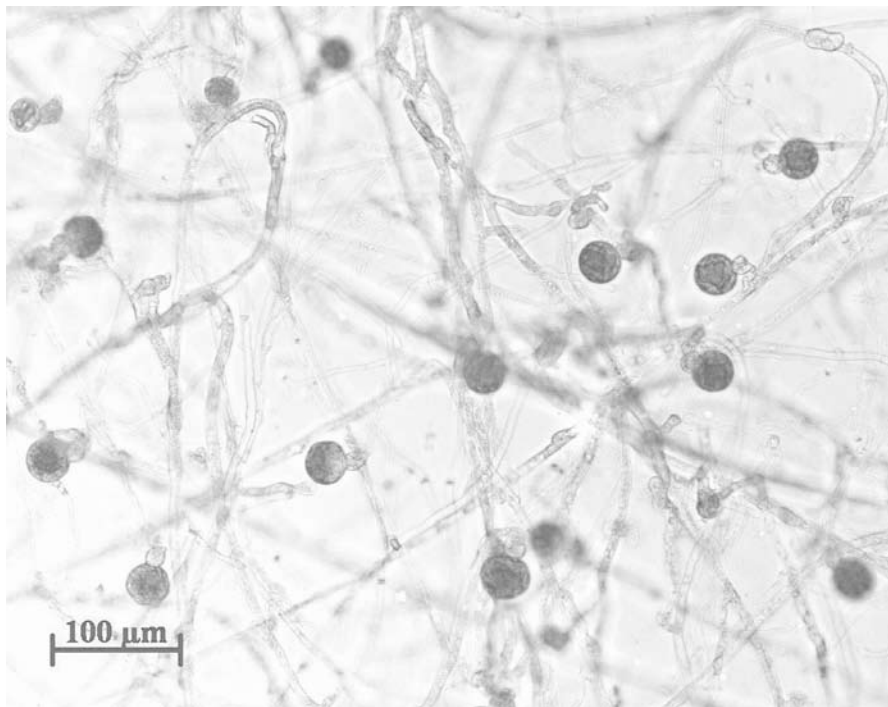


Figure 1. Oospore formation after pairing of A1 and A2 strain of *P. infestans* in vitro culture.

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. Determinations for 50 isolates in 2002-2003 were conducted at Cornell University by using the tester strains US970001 for the A1 mating type and US940480 for the A2 mating type (I). In the years 2004-2007 the tester isolates were the same as those described by Lehtinen *et al.* 2007 (II, III, IV).

3.3. Genotypic markers

3.3.1. RFLP fingerprinting with probe RG57

RFLP analysis (I, II) was performed using the RG57 probe (Goodwin *et al.*, 1992, a). This probe recognizes a dispersed, moderately repetitive and highly polymorphic DNA element that allows the characterization of up to 30 bands in a single hybridization experiment (Fry *et al.*, 1992; Goodwin *et al.*, 1992, a). 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 *Eco*RI, and 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 labeled dATP. For detection, the membranes were washed once in 2X SSC, 0.1 % SDS for 5 minutes followed by 10-minute washes in 1X SSC, 0.1% SDS and then in 0.1X SSC, 0.1% SDS, respectively (all washes at 65°C), then followed by autoradiography (Goodwin *et al.*, 1992, a). 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).

3.3.2. Mitochondrial DNA haplotype

The mitochondrial DNA (mtDNA) haplotype was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (I, II, IV), a variation of the method described by Griffith and Shaw (1998). Primer pairs 1 (F1- GCAATGGG-TAAATCGGCTCAA; R1- AAACCATAAGGACCACACAT) and 2 (F2 - TTCCCTTTTGTCCCTCTACCGAT; R2 - GCTTATGCT-TCAGTTGCTCAT) designed by Griffith and Shaw (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 MgCl₂ (50 mM), 5 µl of dNTPs (2mM), 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 90s. 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).

3.4. Trials of late blight control

Field experiments were arranged at the Jõgeva Plant Breeding Institute in 2003-2005 (V). Two potato varieties differing in resistance to late blight were used: moderately resistant Anti in 2003 and 2004 and susceptible Asterix in 2005. In all three years, two different treatment regimes were used: untreated, where fungicides were not applied and routine treatment where the two first sprayings were made with protectant fungicide Ridomil Gold MZ 68 WG (metalaxyl-M 40, mancozeb 640) 2.5 kg/ha at 10-12 day intervals starting from row closing, and the next treatments were made with the eradicant fungicide Shirlan (fluazinam 500) 0.4 l/ha at 10 day intervals. Trials were laid out according to randomized block design in three replications. The plot size was four rows (7.5*0.7 m). To avoid possible neighbouring effects in border rows, late blight was estimated and the crop was harvested only from the two middle rows. Metalaxyl containing fungicide was applied twice in all three years (V). Late blight infection was assessed twice a week in the plots according to 0-100% scale.

3.5. Data analysis

The isolates were characterized by a multilocus genotype consisting of DNA fingerprint bands, mtDNA haplotype and mating type (I, II). Forty-six isolates were characterized as multilocus genotypes consisting of mating type, mtDNA haplotype and virulence phenotype (IV). Genotypic diversity as well as race diversity was calculated with the normalized Shannon diversity index (Sheldon, 1969): $H_s = - \sum g_i \ln g_i / \ln N$, where g_i is the frequency of the i th multilocus genotype and N the sample size. The normalized index ranges from 0 (there is 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 and years were tested using a logistic analysis (GENMOD procedure in SAS) with a multi-

nomial response variable (A1, A2, or both) (I, II, III, IV). To determine the differences between years (I), only two locations (Jõgeva and Ingliste) were included because other locations were sampled only in one year (I). Similar analyses were performed to compare the proportions of different mating types, haplotypes and isolates resistant to metalaxyl between cropping systems (small and large scale conventional fields and organic fields), i.e. all studied fields were assigned to one of these three groups (IV). 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 years (I, II, IV, V), and also between different haplotypes (I, II). Logistic analyses were also used to test for associations between each of the polymorphic RG57 bands and metalaxyl sensitivity, mating type and mtDNA haplotype (I, II).

The dependence of specific virulence (percent of isolates that show virulence against particular R-genes) on years, sites, field types and R-genes was analyzed with type III ANOVA (I, II, IV). “Site” was treated as a random variable and nested within “field type”. Tukey HSD post-hoc tests ($\alpha = 0.05$) were applied to find specific differences between sites and R-genes. In all analyses, “year” was treated as a categorical variable.

Separate logistic analyses were used to test the dependence of mating type on haplotype and race prevalence (unique vs. prevalent), and the association between virulence complexity (average number of R-genes overcome) and resistance to metalaxyl (IV). The dependence of virulence complexity on cropping system was analysed with one-way ANOVA, as were the differences in the Shannon index values between cropping systems (IV).

4. RESULTS

4.1. Characterisation of Estonian population

4.1.1. Phenotypic characterisation

4.1.1.1. Metalaxyl resistance

Metalaxyl resistance was studied during six years. In the earlier study during 2002-2003 (I, V), 30% were resistant, 51% intermediate and 19% sensitive to metalaxyl (Table 2, I). The difference between the two years was not significant ($\chi^2 = 2.43$, $df = 1$, $p = 0.1192$). All sites had isolates with some insensitivity (intermediate and/or resistant), with the samples from Ingliste 2003 and Kärü 2003 containing only resistant and intermediate isolates (Table 3, I). There were no significant differences among sites (data from $\chi^2 = 1.31$, $df = 4$, $p = 0.86$). Among metalaxyl-resistant isolates, 69% were A1 mating type and 31% were A2 mating type. The level of metalaxyl resistance did not depend significantly on the mating type ($\chi^2 = 2.08$, $df = 1$, $p = 0.15$).

In later period (2004-2007) collected isolates (II, V), 37% of isolates were resistant to metalaxyl, 25% were intermediate and 38% were classified as sensitive. The proportion of metalaxyl-resistant isolates differed between sites ($\chi^2 = 42.20$, $df = 11$, $p < 0.001$) and years ($\chi^2 = 61.57$, $df = 3$, $p < 0.001$). The proportion of metalaxyl-resistant isolates ranged from 0 to 81% depending on site (Figure 1, V). In 2004, 56% of all isolates were resistant to metalaxyl, whereas in 2007, most isolates (Table 2, II) were sensitive to metalaxyl. Metalaxyl-resistant strains were absent from only three sites, where metalaxyl fungicide was not used. Significant differences in metalaxyl resistance between potato field types (large scale, small scale and experimental fields) were not observed ($\chi^2 = 0.05$, $df = 1$, $p = 0.82$) (II). Metalaxyl-resistant isolates were predominant among isolates collected from the experimental field in 2004 (67%) and the conventional fields in 2004 and 2005 (60%) where metalaxyl was used (V). There was a strong association between metalaxyl resistance and sites where metalaxyl-containing fungicides had been applied ($\chi^2 = 9.24$, $df = 1$, $p = 0.0024$). Over four years, from crops known to have been sprayed with fungicide containing metalaxyl, 48% of isolates were resistant, while isolates collected from potatoes which had not been metalaxyl-treated, only 33% of isolates were resistant (Table 3, II). Of the metalaxyl-resistant strains,

63% were A1 mating type, 35% were A2 mating type and 2% were self-fertile. Differences between metalaxyl resistance and mating type were not significant ($\chi^2 = 1.99$, $df = 2$, $p = 0.36$).

In late blight control trials, the foliage of untreated plots was completely destroyed by the end of the growing season in all trial years. Even the late blight infection started in 2003 and 2004, both in the middle of July, the late blight pressure was extremely high in 2004. In 2005, the epidemic started almost one month later. Metalaxyl provided high protection in 2003 and 2005, but showed lowered efficacy only in conditions of extreme late blight pressure in 2004 when the foliage was infected during the period when metalaxyl was used (Figure 2, V).

4.1.1.2. Virulence (Pathotypes)

All known virulence factors (to overcome genes R1-R11) were found in two collections (I, II). In both studies (I, II) isolates with virulence to differentials with R1, R3, R4, R7, R10 and R11 were common. In 2002-2003 (I) the virulence to R5 ($5\% \pm 2.4$ SE) and to R9 ($14\% \pm 7.2$ SE) were somewhat rare ($F_{(10,66)} = 15.89$, $p < 0.0001$) (Figure 2, I). In 2004-2007, least frequent was virulence against R9 (3%) and virulence against R5 was also infrequent (17%) (Figure 1, II). Most of the isolates in both studies (90-96%) (I, II) were able to overcome four or more R-genes (Table 4, I, Table 4, II).

The most common pathotype were in both periods (I, II) 1.3.4.7.10.11 (Table 4, I, Table 4, II). In 2002-2003, sixty-six pathotypes detected among 101 isolates (Table 4, I). In 2004-2007, 87 pathotypes were found among 432 isolates (Table 4, II). Whereas in the earlier study (I) three most common pathotypes made up only 17% of the isolates tested, then in the later collection (II), four most common pathotypes made up almost half (46%) of the isolates. The collection in 2002-2003 (I) was characterised with the high extent (>49%) of unique pathotypes (detected only once) (Table 4, I). In the later collection in 2004-2007 (II) only 13% of pathotypes were detected once (Table 4, II). The mean number of virulences per isolate in the earlier study (I) was 6.3 and in later study 6.9 (II). Variation was high, ranging from 4.3 for isolates from Vönnu 2003 (Table 5, I) to 8.9 in isolates from Jõgeva 2005 (Table 5, II). The normalized Shannon diversity index in 2002-2003 (I) was very high (0.92) in comparison with results from the later study (II) (0.54).

In 2002-2003 (I), 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 (I) ($F_{(1,10)} = 8.30$, $p = 0.06$). In the later collection (II), we found significant differences in the frequencies of specific virulences among sites and years ($F_8 = 9.41$, $p < 0.001$) and virulences to specific R-genes also differed ($F_{10} = 234.31$, $p < 0.001$). No main effect of cropping systems was detected (II).

4.1.1.3. Mating type

In the earlier study in 2002-2003, 60% were A1 and 40% were A2 (Table 2, I). A1 mating type individuals were detected in six of the seven samples, and A2 mating type individuals were identified in four samples (Table 2, I). There were three samples that contained both A1 and A2 mating type individuals (Table 2, I). However, among the five sites in 2003, there were significant differences ($\chi^2 = 18.54$, $df = 4$, $p < 0.001$) in the proportions of A1 and A2.

Among the later period collected isolates (II) 64% were A1 mating type, 33% were A2 mating type and 3% were self-fertile. Both mating types were found at each sampling site (25 sites). The frequency of A2 ranged from 3% to 71%. In 2004 and 2007, almost half (44% and 48%, respectively) of the tested isolates were of the A2 mating type. The proportion of the A2 mating type decreased from 44% in 2004 to 13% in 2006 and increased again to 48% in 2007. There were considerable differences between sites ($\chi^2 = 28.68$, $df = 10$, $p = 0.0014$) and years ($\chi^2 = 26.28$, $df = 3$, $p < 0.001$) in the occurrence of the two mating types. The proportion of A2 mating type was higher among isolates collected from small scale conventional fields (39%) than among those from the large scale conventional fields (26%) ($\chi^2 = 7.05$, $df = 1$, $p = 0.0079$).

Results of the longer term study (III) in one location (Jõgeva) showed that even the frequencies of the A1 mating type (59%) and the A2 mating type (38%) were similar to those found earlier (I, II, III), although considerable differences ($\chi^2 = 45.74$, $df = 12$, $p < 0.001$) were found in the proportions of A1 and A2 between years (III). The frequency of A2 mating type was quite high in 2001, 2003, 2004, 2005 and 2007 (41-71%) (Figure 1, III), whereas the A2 mating type was not found in 2002 (Figure 1, III) and the frequency of A2 mating type was particularly low (3%) in 2006, so that the mean proportion of A2 mating type decreased.

4.1.2. Genotypic characterisation

4.1.2.1. RFLP with the RG57 probe

Eight bands were not detected (bands 4, 11, 12, 15, 17, 19, 22, 23) (Figure 2) and four occurred in all isolates (bands 13, 14, 24, 25) (Table 2, I; Table 6, II). The remaining 13 bands were polymorphic (bands 1, 2, 3, 5, 6, 7, 8, 9, 10, 16, 18, 20, 21). A total of 38 RG57 fingerprints were detected, but five genotypes (I, II, III, XX and XXII) (Table 2, I; Table 6, II) accounted for more than half of the isolates (57%). Twenty-five unique fingerprints were detected at 13 sites, but mostly among isolates collected in 2003 and 2005 (Table 2, I; Table 6, II).

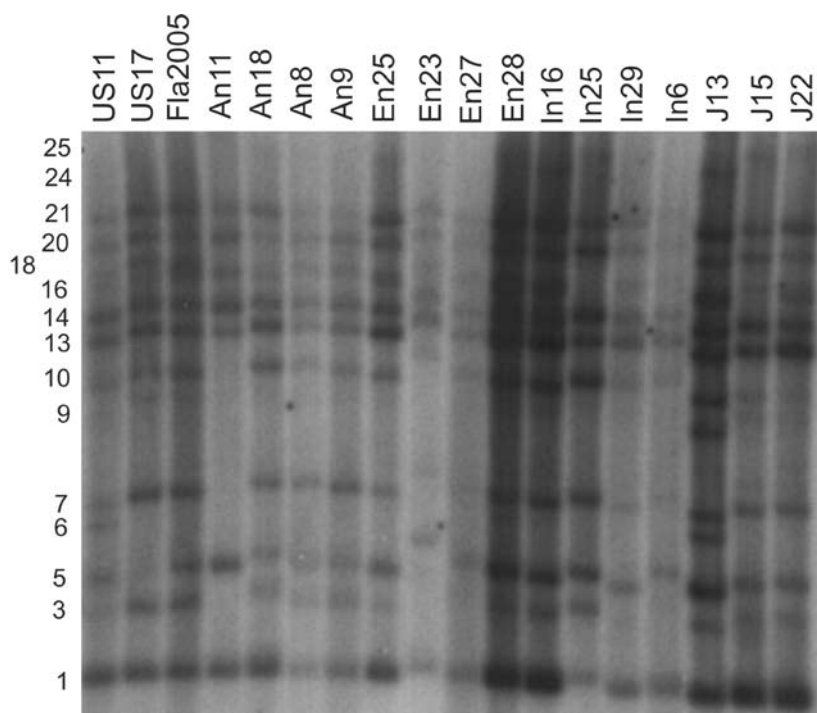


Figure 2. Multilocus RFLP analysis with the RG57 probe of two standard isolates from USA (genotypes US-11 and US-17) and 15 *P. infestans* isolates collected in Estonia from 2004. The fragments indicated on left correspond to those used by Goodwin *et al.* (1992a).

4.1.2.2. Mitochondrial DNA haplotype

Two mitochondrial haplotypes were detected among isolates collected in 2002-2003. Twenty-three were haplotype Ia (46%) and 27 were IIa haplotype (54%). These haplotypes occurred in both A1 and A2 individuals (Table 2, I).

In the later period study (II), of the four mitochondrial DNA haplotypes which are known to be detected, three were found (Figure 3). Among 57 tested isolates, 30 were Ia haplotype (51%), 24 were IIa haplotype (42%) and 3 were IIb haplotype (7%). Of the 37 A1 isolates, 20 were Ia, 14 were IIa and 3 were IIb (Table 6, II). Of the 20 A2 isolates, 10 were Ia and 10 were IIa. There were differences in the frequency of Ia and IIa between sites ($\chi^2 = 40.95$, $df = 26$, $p = 0.031$).

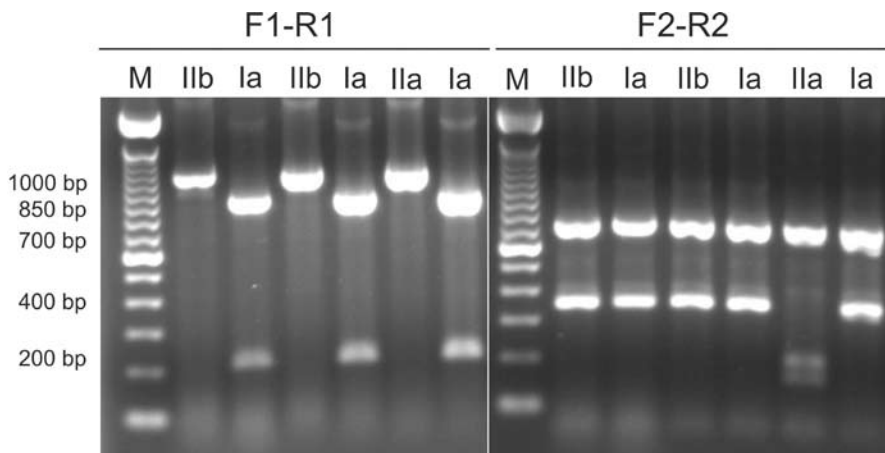


Figure 3. Restriction enzyme digestion of PCR products amplified from the Ia, IIa and IIb haplotypes of two standard isolates from USA (belonging to genotypes US-11, US-17) and four isolates of *P. infestans* collected from Estonia in 2005. Lanes marked M contain DNA molecular weight marker (1-kb ladder).

4.1.2.3. Multilocus genotypes

Fifty-five multilocus genotypes were identified, of which 45 (86%) were detected only once, most collected from Jõgeva (either 2002, 2003 (Table 2, I) or 2005 (Table 6, II)). All belonged to the 'new' population of the pathogen that displaced the US-1 clonal lineage (A1 mating type, Ib mtDNA haplotype) according to Spielman *et al.*, 1991; Lebreton & Andrivon, 1998.

Genotypic diversity measured by the normalized Shannon index was 0.76 (I) and 0.79 (II). Four genotypes were common in the Estonian samples: EE-2 was found 13 times; EE-1 was found 8 times, EE-7 was found 6 times; and EE-4 was found 5 times. Only three multilocus genotypes (EE-1, EE-2 and EE-3) were detected in all four years (Table 2, I; Table 6, II).

4.1.2.4. Associations between markers

Most of RG57 fingerprints were not associated with a single mtDNA haplotype (Table 6, II). There was no association between metalaxyl resistance and mtDNA haplotype ($\chi^2 = 1.66$, $df = 3$, $p = 0.65$) (II). Of the 25 polymorphic RG57 bands, one polymorphic band (16) was significantly associated with metalaxyl tolerance ($\chi^2 = 8.28$, $df = 2$, $p = 0.016$) (II). No associations were found between RG57 fingerprints (I, II), metalaxyl resistance or mating types (I, II, IV).

4.2. Impact of crop management practices

4.2.1. Metalaxyl resistance

In the study where different cropping systems were compared (IV), a total of 49% of the isolates were resistant to metalaxyl, 34% were intermediate and 17% were classified as sensitive. Considerable differences between potato cropping systems were observed ($\chi^2 = 23.75$, $d.f. = 2$, $p < 0.0001$). In particular, in large scale conventional fields, 66% of tested isolates were resistant to metalaxyl, while in small scale farm fields 26% and in organic fields only 14% of the isolates were resistant (Table 3, IV). There were no differences between collection years ($\chi^2 = 0.98$, $d.f. = 1$, $p = 0.42$). Of the metalaxyl resistant strains, 65% were A1 mating type, 30% were A2 mating type and 5% were self-fertile; however, the association between metalaxyl resistance and mating type was not significant ($\chi^2 = 3$, $d.f. = 1$, $p = 0.083$).

4.2.2. Virulence

Of the isolates from the survey of cropping systems (IV) nearly all were virulent on differentials with genotypes R1, R3, R4, R7, R10 and R11. Virulence factor 9 (1%) was rare and factors 5 (10%) and 8 (10%) were relatively rare (Fig 1, Table 4, IV). During the two year period, an in-

crease in virulence factors 2, 5, 8 and 9 was noticed (factor 2: $\chi^2 = 10.95$, d.f. = 1, $p = 0.0009$; factor 5: $\chi^2 = 9.38$, d.f. = 1, $p = 0.0022$; factor 8: $\chi^2 = 16.03$, d.f. = 1, $p < 0.0001$; factor 9: $\chi^2 = 5.55$, d.f. = 1, $p = 0.019$). After applying a Bonferroni correction (since 11 comparisons were made), only the differences in virulence factors 2, 5 and 8 remain significant.

Thirty-eight races were detected (Table 5, IV). The two most common races made up 70% (Table 5, IV) of the isolates tested. The overall virulence complexity (average number of R-genes overcome) was 6.7 (Table 5, IV). Virulence complexity was the highest in organic farms (7.3). Complex races predominated in organic fields, but were less common in small and large scale conventional fields ($F_{(193)} = 8.49$, $p = 0.00029$). The overall normalized Shannon diversity index was 0.38 and differed significantly between cropping systems ($F_{(2)} = 23.89$, $p = 0.0028$). This index was as high as 0.71 in large scale conventional fields, but much lower in small scale (0.13) and organic fields (0.18).

4.2.3. Mating type

Among isolates collected from different cropping systems (IV) 57% were A1 mating type, 41% were A2 mating type and 2% were self-fertile. At 11 of the 12 fields, both A1 and A2 mating types were detected. The proportion of the A2 mating type increased abruptly from 28% in 2004 to 54% in 2005 ($\chi^2 = 11.87$, d.f. = 1, $p = 0.0006$). There were further differences between cropping systems ($\chi^2 = 9.60$, d.f. = 2, $p = 0.0082$), the proportion of A2 being highest in organic fields and lowest in large scale conventional fields (Table 2, IV).

4.2.4. Mitochondrial DNA haplotype

Three mitochondrial haplotypes (Ia, IIa and IIb) were detected among 66 isolates tested. Two isolates of haplotype IIb were found from large scale conventional fields. The majority of isolates were haplotype IIa (74%) and minority were Ia (23%). No significant differences were found in the frequencies of Ia and IIa between years ($\chi^2 = 1.46$, d.f. = 1, $p = 0.23$). However, differences between cropping systems were observed ($\chi^2 = 8.38$, d.f. = 2, $p = 0.015$), with the highest proportion of IIa in large scale conventional fields and lowest in organic fields. Interestingly, in the latter, only one haplotype (Ia) was detected (Table 6, IV). There was no association between mating type and haplotype ($\chi^2 = 0.76$,

d.f. = 1, $p = 0.38$). There were no differences between isolates taken early *vs* late during the season, in the prevalence of different haplotypes; nor in the proportions of mating types, metalaxyl resistance or the number of R-genes overcome (statistics not shown).

4.2.5. Multilocus genotypes

Eighteen multilocus genotypes were identified among 46 isolates. In large scale conventional fields, most of the isolates were unique, i.e. found only once (Table 7, IV). Genotypic diversity measured by the normalized Shannon index was 0.64 and differed significantly between cropping systems ($F_{(2)} = 41.76$, $p < 0.001$). In large scale conventional fields, this index was 0.97, in small scale fields 0.53, and, in organic fields only 0.28.

5. DISCUSSION

5.1. Characterisation of *Phytophthora infestans* Estonian populations

In the present thesis, newly established studies in Estonia, on the phenotypic and genotypic characteristics of *P. infestans* isolates, collected from potato in 2002-2003 and more detailed characterization in 2004-2007 are reported and results compared with those from other European countries. For phenotypical characterisation, metalaxyl sensitivity, virulence and mating type were used. RFLP fingerprint (Goodwin *et al.*, 1992, a) and mtDNA haplotype (Griffith & Shaw, 1998) were used as molecular markers.

5.1.1. Phenotypic characterisation

The average proportion of isolates that were sensitive (34%), intermediate (33%) and resistant (33%) to metalaxyl in the Estonian population, were almost equal. The proportion of metalaxyl-resistant isolates has fluctuated considerably in recent years 2002-2007 (I, II, IV, V), from about 20-35% in 2002 and 2003 (V) it increased to 56% in 2004. This peak in 2004 was followed by a decrease among isolates collected in 2006 and 2007, when metalaxyl-resistant isolates were, again, in the minority (Table 2, II). During the peak of metalaxyl resistance (2004-2005), the epidemics started earlier and were more severe than those of 2002-2003 (I). The reason for the increase in resistance may have been the intensive use of metalaxyl in Estonia against the heavy late blight pressure during the peak years. Part of the reason for the subsequent decrease in resistance could be that, in 2007, the weather conditions were not favourable for late blight development and the pathogen occurred quite late in the season, so farmers/growers did not treat the crop as frequently and did not use metalaxyl fungicides as often as in other years. Although the proportion of metalaxyl-resistant isolates fluctuates from year to year it is still clear that this proportion has remained substantially high throughout the whole monitoring period. As mentioned above, the most likely reason for the fluctuations is the changing intensity of the use of metalaxyl-containing fungicides. The situation was very similar to that in Finland and Norway in the early and mid 1990s when approximately 60% of the isolates were resistant to metalaxyl (Hermansen *et al.*, 2000) and the use of phenylamide fungicides was very common (Han-

nukkala *et al.*, 2007). Thereafter, the situation changed and the proportion of metalaxyl resistant isolates decreased from 40% in 1997 to 17% in 2000 (Lehtinen *et al.*, 2007).

Interesting changes in the percentage of Estonian isolates displaying intermediate resistance to metalaxyl were found. The proportion of isolates with intermediate resistance was high (39%) during the period 2002-2006 (II) but by the year 2007, the proportion had decreased to 6%. A similar situation was noted in Great Britain by Cooke *et al.* (2003) and Day *et al.* (2004).

While metalaxyl resistance was found to be more frequent among A1 than A2 isolates in 2002-2003 (I), the difference between A1 and A2 mating types in regard to metalaxyl resistance was not dramatically large, and our limited samples preclude further speculation. During the 1990s, metalaxyl resistance was clearly associated with the A1 mating type in many European countries (Hermansen *et al.*, 2000; Day *et al.*, 2004; Cooke *et al.*, 2006; Lehtinen *et al.*, 2007). Among isolates collected in 2004-2007 from Estonia this was not obvious anymore and the proportion of metalaxyl resistant isolates was almost the same between A1 and A2 mating type isolates. 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.

Metalaxyl provided high protection under moderate pathogen pressure at late blight control trials (V), but showed lowered efficacy only in conditions of extreme late blight pressure in 2004 when the foliage was infected during the period when metalaxyl was used. Results showed that, in spite of the occurrence of resistant strains, the use of metalaxyl containing fungicides is still effective (V). However, the temporal variation in our data suggests that intensive treatment can lead to increased resistance. Therefore metalaxyl could be used effectively for control of potato late blight a maximum of two times at the start of the fungicide treatments. By following the application instructions for metalaxyl fungicides, it is possible to restrict the selection for metalaxyl-resistant strains and thus avoid the reduction of the efficacy of the fungicide (V). As long as metalaxyl is used according to the recommendations in Estonia it is unlikely that metalaxyl-resistant isolates will completely displace sensitive isolates (V).

Besides testing for metalaxyl-resistance, isolates were also tested for virulence phenotypes. Isolates of *P. infestans* virulent to differential factors R1, R3, R4, R7, R10 and R11 dominated in Estonian populations in all study years: 2002-2007 (I, II, IV). Populations with similar results have been described in the 1990s in Finland and Norway (Hermansen *et al.*, 2000), Finland (Lehtinen *et al.*, 2007, Lehtinen *et al.*, 2008), Denmark and Sweden (Lehtinen *et al.*, 2008), France (Knapova & Gisi, 2002; Pilet *et al.*, 2005) and Scotland (Cooke *et al.*, 2003). Estonian isolates have medium frequency of virulence against R2 (40-50%), R6 (28-48%) and R8 (37-53%) (I, II, IV) which is most similar to that observed in Denmark and Sweden (Lehtinen *et al.*, 2008). The Estonian population of *P. infestans* is characterised with low frequency of differential factors R5 (5-17%) and R9 (3-14%) in both periods (I, II) in Estonia. Virulent isolates on differentials with R-gene 9 are very rare in most populations, found further away from the Estonian population, as from Poland (Śliwka *et al.*, 2006; Lebecka *et al.*, 2007).

The Estonian race structure in 2002-2003 (I) was highly diverse and complex. Most races were unique, appearing only once (Table 2, I), and the three most common pathotypes (Table 2, I) comprised only 17% of the population. On the contrary, in the larger isolate collection in 2004-2007 (II) the four most common pathotypes formed almost a half (46%) of all isolates (Table 4, II). The race 1.3.4.7.10.11 was the most common among Estonian isolates in both studies (I, II, IV) as in Finland, Norway, Denmark and Sweden (Hermansen *et al.*, 2000, Lehtinen *et al.*, 2007, Lehtinen *et al.*, 2008), in France and Switzerland (Knapova & Gisi, 2002), in Poland (Zimnoch-Guzowska, 1999) and in Austria (Avenidaño Córcoles, 2007).

Complex races are common in Estonian populations (I, II, IV), as in Polish (Sujkowski *et al.*, 1996; Śliwka *et al.*, 2006; Lebecka *et al.*, 2007) and Russian populations of *P. infestans* (Elansky *et al.*, 2001; Vedenyapina *et al.*, 2002). The mean number of virulence genes per isolate increased from 6.3 in 2002-2003 to 6.9 in 2004-2007. A similar increase has also occurred in Finland and Norway (Lehtinen *et al.*, 2008).

Pathotype diversity calculated by the normalised Shannon diversity index showed very high values (0.92) in 2002-2003 (I), analogous to the race structure. In the larger sampling collection of 2004-2007 (II), the index value showed the same range as other European populations in Aus-

tria (0.56) (Avendaño Córcoles, 2007), in Poland (0.53) (Sujkowski *et al.*, 1996), Finland (0.35) and Norway (0.44) (Hermansen *et al.*, 2000). Still, results of these two periods (I, II) illustrate considerable pathotypic diversity (0.73) in the Estonian population of *P. infestans*.

Mating type was chosen as one of the phenotypical markers for characterisation of *P. infestans*. In 1984, Hohl and Iselin reported the discovery of isolates with the A2 mating type for the first time in Europe among isolates collected in Switzerland in 1981. After that, the A2 mating type isolates spread rapidly over Europe. In Estonia, the A2 mating type was detected for the first time in 1987 by researchers of the All Union Institute of Plant Pathology (Vorobyeva *et al.*, 1991).

Investigations during both periods 2002-2003 (I) and 2004-2007 (II) indicated the presence of both mating types together in most of the studied sites, suggesting the occurrence of sexual reproduction in Estonian populations (I, II).

In 2002-2003, the ratio of A1 and A2 mating types was 60:40 (I) and in extensive analysis in 2004-2007 the ratio was 64:33, the proportion of self-fertile isolates was 3% (II). These results indicate that the mean proportion of the A2 mating type in Estonia was clearly higher than in most other European countries (Lebreton & Andrivon, 1998; Brurberg *et al.*, 1999; Hermansen *et al.*, 2000; Bakonyi *et al.*, 2002a; *et al.*, 2002b; Cooke, *et al.*, 2003; Cooke *et al.*, 2006; Lehtinen, *et al.*, 2007) and was similar to that in Austria (Avendaño Córcoles, 2007), the Netherlands (Zwankhuizen *et al.*, 2000), Hungary, the Czech Republic (Mazakova *et al.*, 2006), Poland (liwka *et al.*, 2006, Lebecka *et al.*, 2007), Russia (Leningrad region) (Vedenyapina *et al.*, 2002) and Nordic countries in recent years (Lehtinen *et al.*, 2008). A higher proportion of the A2 mating type has been found in certain years and/or regions in Germany, Poland, the Netherlands, Finland and Norway (Schöber & Turkensteen, 1992; Sujkowski *et al.*, 1994; Zwankhuizen *et al.*, 2000; Hermansen *et al.*, 2000; Lehtinen *et al.*, 2007). Study indicated that, in Estonia, A2 and A1 isolates often occurred in the same field, suggesting that sexual reproduction is theoretically possible (see also Turkensteen *et al.*, 2000). As our results indicated, on several (41%) studied fields, the two mating type ratio is close to 1:1, that also indicate higher possibilities for sexual reproduction in Estonia. The situation has also dramatically changed in the UK, where the *P. infestans* population used to consist mostly of the

A1 mating type but has shifted to the A2 mating type strain since 2007 (Lees *et al.*, 2009). Differences in the A1/A2 ratio between sites, regions and years in Estonia in both periods (I, II) were considerable.

The main reason for studying mating types of Estonian isolates of *P. infestans* was to verify the potential of *P. infestans* sexual reproduction in Estonia. Both mating types were detected in almost all fields studied (I, II, III, IV). The practice of continuous potato cropping in small scale conventional fields increases the risk of oospore-derived infections and may cause more early attacks and consequent yield loss (II). The presence of both mating types in the same field indicates that oospores would be produced in potato foliage (and may change pathogen epidemiology substantially). Indeed, in the year 2004, the disease symptoms of infected potato plants indicated that the infection was probably caught from oospore-contaminated soil (II). Therefore more emphasis needs to be put on crop rotation (Turkensteen *et al.*, 2000).

5.1.2. Genotypic characterisation *Phytophthora infestans* Estonian populations

The RG57 fingerprints showed high genotypic diversity since 38 different RFLP genotypes were identified among 107 isolates collected in 2002-2003 (I) and 2004-2005 (II). Most of the 38 RG57 fingerprints in 2002-2005 are apparently unique to Estonia because they have not been reported elsewhere. This high genetic variability could imply that sexual reproduction plays an important role of pathogen reproduction in Estonia (II) as in Nordic, Eastern and Central European countries. However, seven fingerprints (I, IV, V, XXI, XXIII, XXXV) (Table 5, I; Table 6, II) were identical with fingerprints identified in Russia, Norway, Great Britain, Finland, the Netherlands and Northern Ireland (Table 5, I; Table 6, II). 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) (Table 6, I). 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). The fingerprint XX was identical with the Dutch fingerprint NL-86 (Zwankhuizen *et al.*, 2000), the fingerprint XXI with the Russian fingerprint MO-5

(Elansky *et al.*, 2001), XXIII was identical with the Russian fingerprint MO-17 (Elansky *et al.*, 2001) and the British fingerprint RF015 (Day *et al.*, 2004) XXXV was identical with the British fingerprint RF006 and the most common Northern Ireland fingerprint NI-1 (Day *et al.*, 2004, 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.

The mitochondrial DNA haplotypes were determined to see whether the 'old' population (US-1 clonal lineage) with the Ib haplotype had been shifted by the new diverse population or whether the 'old' clonal lineage (US-1) was still present. In first survey in 2002-2003, the Ia and IIa haplotypes were detected. In the later period, 2004-2005, three mtDNA haplotypes (Ia, IIa and IIb) were found among the tested isolates (I, II). The Ib haplotype, associated with the old clonal *P. infestans* populations, was not found (I, II). The rare haplotype IIb was found for the first time in Estonia in 2004 (II). Interestingly, among isolates collected from metalaxyl-treated crops the extent of the IIb haplotype, which is rare in Europe, was 23% (II). In both studies (I, II), both haplotypes were found in almost equal proportions. For example in 2002 to 2003, the ratio of Ia and IIa haplotypes was 46:52 and in the period 2004-2005, the ratio of three haplotypes Ia, IIa and IIb was 51:42:7. In Finland (Lehtinen *et al.*, 2007), Eastern Europe e.g. Hungary (Nagy *et al.*, 2006), Leningard region in 2003-2004 (unpublish data from A. Filippov), Moscow region in Russia (Elansky *et al.*, 2001), the proportion of IIa haplotype had a higher frequency than Ia haplotype isolates. In contrast, in Poland (Lebecka *et al.*, 2007), Austria (Avendano Corcoles, 2007), Germany (Rullich & Schöber-Butin, 2001), Scotland (Cooke *et al.*, 2003), France (Lebreton *et al.*, 1998) Ia haplotype was more prevalent than the IIa haplotype. By inference of these reports we suggest that the Estonian population of *P. infestans* is related more to the populations from Eastern and Northern Europe than to those from Western Europe.

5.2. Impact of crop management practices to Estonian population of *Phytophthora infestans*

Metalaxyl resistant isolates were found four times more frequently (Table 3, IV) from large scale conventional fields than from organic fields. This difference could be explained by the use of metalaxyl products in large

scale conventional fields, even though no significant differences were detected between large scale conventional fields treated and untreated with metalaxyl (IV). Further differences between crop systems (IV) were evident in the resistance of isolates to metalaxyl fungicides.

Resistance to metalaxyl was more often found in potato fields known to have been sprayed with phenylamide-containing fungicides (II), 48% of isolates were resistant, whereas from fields which had not been treated with phenylamide, only 33% of isolates were resistant.

Race diversity calculated by the normalized Shannon diversity index showed low value (0.38) (IV), but differed strongly among crop systems. The diversity index was much higher (IV) among isolates collected from large scale conventional fields (0.71) than from organic (0.18) and small scale conventional fields (0.13). This result is particularly puzzling because, unlike smaller farms, large scale farms used certified potato seed tubers and applied rotation. Somewhat contra-intuitively, the most complex races were actually more common in inorganic fields. Perhaps this result can be explained by the reason that large scale conventional farms import potato seed from the Netherlands, Germany and Denmark and grow mostly foreign varieties. Particularly, in the Netherlands high numbers of rare genotypes have been detected (Turkensteen *et al.*, 2000) and oospores have been shown to play an active role in the epidemiology of *P. infestans* in the Netherlands (Turkensteen *et al.*, 2000). In while, small scale conventional and organic producers use seed potatoes from Estonian seed potato growers and are likely to contain only the locally prevalent pathogen genotypes (see discussion below, page).

The presence of both mating types in the same field indicates the possibility of oospore production in potato foliage (Turkensteen *et al.*, 2000). Both mating types were detected from nearly all sites (IV), with the single exception of an organic field in 2004. Different results have been reported from Finland (Lehtinen *et al.*, 2007), the Netherlands (Zwankhuizen *et al.*, 2000) and Scotland (Cooke *et al.*, 2003), where the co-occurrence of both mating types was more common in organic fields.

In organic fields, 62% of isolates were A2 mating type whereas in large scale conventional farm fields only 31% of isolates were A2 mating type (Table 2, IV). The possible higher prevalence of the A2 mating type and of both mating types found from most fields, together with the lack of

crop rotation may presume higher risk for sexual reproduction in organic fields compared to other crop systems. However, based on our results (IV), differences in the A1/A2 ratio between crop systems can be suggested, even though larger sample sizes are needed to explicitly prove this finding. Similarly, the proportion of A2 mating type was higher among isolates collected from untreated experimental fields (40%) and small scale conventional farm fields (39%) than from large scale conventional fields (26%). Differences in these scales could signify higher genetic diversity among populations collected from experimental and small scale productions, as there is a higher risk of sexual reproduction (II).

Also, the multilocus genotypic diversity was found to be particularly high in large scale conventional productions. A possible explanation could be related to the background of seed potatoes used in different farms. In particular, large scale conventional producers import seed potatoes directly from Central Europe (the Netherlands, Germany and Denmark), after which the tubers are multiplied locally for two years. Small scale conventional and organic producers, on the other hand, use seed potatoes that originate from Estonian seed potato growers and are likely to contain only the locally prevalent pathogen genotypes. The same could explain the higher race diversity found in large scale fields (see above).

Another dissimilarity found between crop systems was the higher prevalence of the generally less common IIa haplotype in large scale conventional fields compared to other field types. The reason for this difference may again be the use of imported seed potatoes by large scale farmers (see discussion above, page). The high proportion of Ia haplotype (74%) in this study differs from the results of the previous study conducted in Estonia (I, II). A higher proportion of Ia haplotype has also been observed in Poland, England, Scotland, Wales, the Netherlands and France (Leberton & Andrivon *et al.*, 1998; Cook *et al.*, 2003; Lebecka *et al.*, 2007).

The markers used were chosen to show mainly phenotypic variability in different crop management practices, with genetic variation characterized by mtDNA haplotypes. In further studies, it would also be informative to use microsatellite markers to detect the specific relationships between phenotypic and genotypic variation, as reported in Guo *et al.*, (2009). Work with SSR markers have started and will be reported in future publications.

5.3. Advice for potato producers

The results of this study (I, II, III, IV, V) suggest that there may be considerable differences between potato crop systems in various aspects of the population structure of *P. infestans* inhabiting the fields. However, it is probable that different management practices, mainly fungicide use but also crop rotation and the source of potato seeds, rather than the crop system *per se*, are behind these differences. Dissimilarities were found in the prevalence of mating types, virulence genes, mtDNA haplotypes and resistance to metalaxyl. These figures were also not always similar to those found in previous studies (I, II) in this or other geographical regions, implying a noticeable spatial and temporal variation in *P. infestans* population parameters. This may imply that growers also need to consider the regional situation to make optimal decisions.

Such differences probably lead to variation in the risk of yield loss. In contrast to the previous assumptions, several aspects of pathogen diversity, such as genotypic diversity, race complexity and the diversity of mtDNA haplotypes appeared to be highest in the large scale conventional fields. On the other hand, the proportion of the novel A2 mating type and virulence complexity were highest in the organic fields. The prevalence of metalaxyl resistance was also highest in the large scale conventional fields. Such differences should not be ignored by producers, and different precautions can be suggested for managing different types of farms. In particular, conventional farmers may benefit from the use of other control methods beside metalaxyl fungicides to limit the spread of resistance in the pathogen population.

Potato late blight is a highly yield-limiting disease on conventional and especially in organic potato production. It is very important for both organic and conventional farmers to adhere to official crop rotation requirements and not grow potatoes continuously. In situations where both mating types (A1, A2) coexist in the same field, the necessary interval between potato crops is at least four years, mainly because of risk of soil-borne infection with oospores. Even in the absence of sexual reproduction, *P. infestans* is highly efficient as a potato pathogen and causes notable yield loss.

For organic producers in Estonia, we suggest the growing of only local (Jõgeva Plant Breeding Institute) more resistant potato varieties.

The habit for conventional producers to grow mostly western European potato varieties, which are well advertised in markets and by the food industry, but unfortunately are susceptible to late blight in Estonian conditions, is very hard to change. However, it is not sufficient to rely on varietal resistance to control late blight, as, in favourable weather, late blight can severely affect these varieties unless they are sprayed with fungicide. Even resistant varieties should be sprayed regularly with fungicides to eliminate infection of tubers. In Estonia, it is not possible to achieve high yield with good quality in conventional production without control against the late blight pathogen (Koppel, 1997). Most important is timing the first preventive spraying. For risk reduction all known agrotechnical techniques must be followed and only certified seed potatoes should be used. Protective copper-based fungicides which could relieve the high pressure of the pathogen and which are used in organic production systems in Europe are prohibited in Estonia.

6. CONCLUSIONS

This study confirms earlier reports (Goodwin *et al.*, 1994; Vorobyeva *et al.*, 1991) that the population of *P. infestans* in Estonia in 2002-2003 (I) and 2004-2007 (II) 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. 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; Sujkowski *et al.*, 1996; Brurberg *et al.*, 1999; Bakonyi *et al.*, 2002a; Day *et al.*, 2004; Nagy *et al.*, 2006; Śliwka *et al.*, 2006; Lehtinen *et al.*, 2008; Lees *et al.*, 2009). Our study indicates that the population of *P. infestans* in Estonia is characterized 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

The data from our studies (I, II) indicate that the Estonian population of *P. infestans* is diverse, having a large number of multilocus genotypes. When comparing the results of these studies (I, II) with those of other studies, it is clear that the population from Estonia has significant similarity to populations in Finland, Norway and Russia. 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 neighboring countries (I, II).

The prevalence of metalaxyl resistance of *P. infestans* displayed considerable temporal fluctuations during the years of study, possibly in connection with the intensity of fungicide use. We predict that, as long as metalaxyl is used according to the recommendations, it is unlikely that metalaxyl-resistant isolates will completely displace sensitive isolates in Estonia (V).

The high and stable frequency of A2 isolates and the occurrence of both mating types on the same field indicates a potential for sexual recombination and spreading of fungicide resistance (I, II, III, IV). More information is needed to clarify the role of oospores in the epidemiology of *P. infestans* in Estonia (II).

The results from the study of crop management practices (IV) suggest that there may be considerable differences between potato crop systems in various aspects of the population structure of *P. infestans* inhabiting the fields. Dissimilarities were found in the prevalence of mating types, virulence genes, mtDNA haplotypes and resistance to metalaxyl. The higher prevalence of the A2 mating type, both mating types found from most fields, and possible lack of crop rotation may presume higher risk for sexual reproduction at organic fields compared to other cropping systems (IV).

The population parameters described in this study were not always similar to those found in previous studies in this or other geographical regions, implying a noticeable spatial and temporal variation in *P. infestans* population parameters. This may imply that managers also need to consider the regional situation to make optimal decisions (III).

REFERENCES

- Anderson, B., Sandström, M. & Strömberg, A. 1998. Indication of soil-borne inoculum of *Phytophthora infestans*. Potato Research, **41**, 305-310.
- Andrивon, D. 1994. Races of *Phytophthora infestans* in France, 1991-1993. Potato Research, **37**, 279-286.
- Avendaño Córcoles, J. 2007. Survey of *Phytophthora infestans* population in Austria based on phenotypic and molecular markers. Vienna, Austria: University of Natural Resources and Applied Life Sciences, PhD thesis.
- Bagirova, S.F. & Dyakov, Yu.T. 1998. Participation of *Phytophthora infestans* oospores in spring epidemics resumption. Sel'skohozyaistvennaya Biologia, **3**, 69-71.
- Bakonyi, J., Ládai, M., Dula, T. & Érsek, T. 2002a. Characterisation of isolates of *Phytophthora infestans* from Hungary. European Journal of Plant Pathology, **108**, 139-146.
- Bakonyi, J., Heremans, B. & Jamart, G. 2002b. Characterization of *Phytophthora infestans* isolates collected from potato in Flanders, Belgium. Phytopathology, **150**, 512-516.
- Brurberg, M.B., Hannukala, A. & Hermansen, A. 1999. Genetic variability of *Phytophthora infestans* in Norway and Finland as revealed by mating type and fingerprint probe RG57. Mycological Research, **12**, 1609-1015.
- Carlisle, D.J., Cooke, L.R., Watson, S. & Brown, A.E. 2002. Foliar aggressiveness of Northern Ireland isolates of *Phytophthora infestans* on detached leaflets of three potato cultivars. Plant Pathology, **51**, 424-434.
- Caten, C.E. & Jinks, J.L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Canadian Journal of Botany, **46**, 329-348.
- Cooke, D.E.L., Young, V., Birch, P.R.J., Toth, R., Gourlay, F., Day, J.P., Carnegie, S F. & Duncan, J. M. 2003. Phenotypic and genotypic diversity of *Phytophthora infestans* populations in Scotland (1995-97). Plant Pathology, **52**, 181-192.

- Cooke, L.R., Carlisle, D.J., Donaghy, C., Quinn, M., Perez, F.M. & Deahl, K.L., 2006. The Northern Ireland *Phytophthora infestans* population 1998-2002 characterized by genotypic and phenotypic markers. *Plant Pathology*, **55**, 320-330.
- Davidse, L.C., Looijen, D., Turkensteen, L.J. & van der Val, D. 1981. Occurrence of metalaxyl-resistance strains of *Phytophthora infestans* in Dutch potato fields. *Netherlands Journal of Plant Pathology*, **87**, 65-68.
- Day, J.P., Wattier, R.A.M., Shaw, D.S. & Shattock, R.C. 2004. Phenotypic and genotypic diversity in *Phytophthora infestans* on potato in Great Britain, 1995-98. *Plant Pathology*, **53**, 303-315.
- Dowley, L.J. & O'Sullivan, E. 1981. Metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. *Potato Research*, **24**, 417-421.
- Drenth, A., Tas, I.C.Q. & Govers, F. 1994. DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. *European Journal of Plant Pathology*, **100**, 97-107.
- Drenth, A., Turkensteen, L. J. & Govers, F. 1993. The occurrence of the A2 mating type of *Phytophthora infestans* in the Netherlands; significance and consequences. *Netherlands Journal Plant Pathology*, **99**(3), 57-67.
- Elansky, S., Smirnov, A., Dyakov, Y., Dolgova, A., Filippov, A., Kozlovski, B., Kozlovskaja, I., Russo, P., Smart, C. & Fry, W. 2001. Genotypic analysis of Russian isolates of *Phytophthora infestans* from the Moscow region, Siberia and Far East. *Phytopathology*, **149**, 605-611.
- Fry, W.E. & Goodwin S.B. 1997. Re-emergence of potato and tomato late blight in the United States. *Plant Disease*, **81**, 1349-1357.
- Fry, W.E., Goodwin, S.B., Dyer, A.T., Matuszak, J.M., Drenth, A., Tooley, P.W., Sujkowski, L.S., Koh, Y.J., Cohen, B.A., Spielman, L.J., Deahl, K.L., Inglis, D.A. & Sandlan, K.P. 1993. Historical and recent migrations of *Phytophthora infestans*: chronology, pathways, and implications. *Plant Disease*, **77**, 653-661.
- Fry, W.E., Goodwin, S.B., Matuszak, J.M., Spielman, L.J., Milgroom, M.G. & Drenth, A. 1992. Population genetics and intercontinental migrations of *Phytophthora infestans*. *Annual Review of Phytopathology*, **30**, 107-129.

- Gisi, U. & Cohen, Y. 1996. Resistance to phenylamide fungicides: A case study with *Phytophthora infestans* involving mating type and race structure. *Annual Review of Phytopathology*, **34**, 549-572.
- Goodwin, S.B., Sujkowski, L.S. & Fry, W.E. 1996. Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and western Canada. *Phytopathology*, **85**, 793-800.
- Goodwin, S.B., Schneider, R.E. & Fry, W.E. 1995. Use of celluloseacetate electrophoresis for rapid identification of allozyme genotypes of *Phytophthora infestans*. *Plant Disease*, **79**, 1181-1185.
- Goodwin, S.B., Cohen, B.A. & Fry, W.E. 1994. Planglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Sciences, USA* **91**, 11591-11595.
- Goodwin, S.B., Drenth, A. & Fry, W.E. 1992a. Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Current Genetics*, **22**, 107-115.
- Goodwin, S.B., Spielman, L.J., Matuszak, J.M., Bergeron, S.N. & Fry, W.E. 1992b. Clonal diversity and genetic differentiation of *Phytophthora infestans* in northern and central Mexico. *Phytopathology*, **82**, 955-961.
- Griffith, G.W. & Shaw, D.S. 1998. Polymorphisms in *Phytophthora infestans*: Four mitochondrial haplotypes are detected after PSR amplification of DNA from pure cultures or from host tissue. *Applied and Environmental Microbiology*, **64**, 4007-4014.
- Hannukkala, A. O., Kaukoranta, T., Lehtinen, A. & Rahkonen, A. 2007. Late-blight epidemics on potato in Finland, 1933-2002; increased and earlier occurrence of epidemics associated with climate change and lack of rotation. *Plant Pathology*, **56**, 167-76.
- Hannukkala, A. & Lehtinen A. 2005. Management of potato late blight, *Phytophthora infestans*, inorganic production. In: E. Ritter, A. Carrascal (eds). 16th triennial conference of the EAPR, Bilbao, Spain.
- Hermansen, A., Hannukkala, A., Hafskjold Nærstad, R. & Brurberg, M. 2000. Variation in populations of *Phytophthora infestans* in Finland and Norway: mating type, metalaxyl resistance and virulence phenotype. *Plant Pathology* **49**, 11-22.

- Knapova, G. & Gisi U. 2002. Phenotypic and genotypic structure of *Phytophthora infestans* populations on potato and tomato in France and Switzerland. *Plant Pathology*, **51**, 641-653.
- Koppel, M. 1997. Muutused kartuli lehemädanikukindluses aastatel 1922-1991. – Kaasaja ökoloogia probleemid. Ajalised muutused Eesti eluslooduses ja keskkonnas. Tartu, lk. 97-101.
- Koppel, M. 1996. Muutused *Phytophthora infestans* populatsioonis ja kartulisortide lehemädanikukindluses. Akadeemilise Põllumajanduse Seltsi toimetised nr. 1, lk. 30-33.
- Lebecka, R., Śliwka, J., Sobkowiak, S., Zimnoch-Guzowska, E. 2007. *Phytophthora infestans* population in Poland. PPO-Special Report no., **12**, 155-159.
- Lebreton, L. & Andrivon, D. 1998. French isolates of *Phytophthora infestans* from potato and tomato differ in phenotype and genotype. *European Journal of Plant Pathology*, **104**, 583-94.
- Lees, A.K., Cooke, D.E.L., Stewart, J.A., Sullivan, L., Williams, N.A. & Carnegie, S.F. 2009. *Phytophthora infestans* population changes: implications. PPO-Special Report no. **13**, 55-60.
- Lehtinen, A., Hannukkala A., Andersson, B., Hermansen, A., Le, V.H., Naerstad, R., Brurberg, M.B., Nielsen, B.J., Hansen, J.G. & Yuen, J. 2008. Phenotypic variation in Nordic populations of *Phytophthora infestans* in 2003. *Plant Pathology*, **57**, 227-234.
- Lehtinen, A., Hannukkala, A., Rantanen, T. & Jauhiainen L. 2007. Phenotypic and genetic variation in Finnish potato-late blight populations, 1997-2000. *Plant Pathology*, **56**, 480-491.
- Malcolmson, J.F. & Black, W. 1966. New R genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica*, **15**, 199-203.
- Mazáková, J., Táborský, V., Zouhar, M., Rysánek, P., Hausvater, E., & Dolezal, P. 2006. Occurrence and distribution of mating types A1 and A2 of *Phytophthora infestans* (Mont.) de Bary in the Czech Republic. *Plant Protection Science*, **42**, 41-48.
- Mayton, H., Smart, C.D., Moravec, B.C., Mizubuti, E.S.G., Muldoon, A.E. & Fry, W.E. 2000. Oospore survival and pathogenicity of single oospore recombinant progeny from a cross involving US-17 and US-8 genotypes of *Phytophthora infestans*. *Plant Disease*, **84**, 1190-1196.

- McLeod, A., Denman, S., Sadie, A. & Denner, F.D.N. 2001. Characterisation of South African isolates of *Phytophthora infestans*. Plant Disease, **85**, 287-291.
- Nagy, Z.Á., Bakonyi J., Virag Som & Érsek T. 2006. Genetic Diversity of the population of *Phytophthora infestans* in Hungary. Acta Phytopathologica et Entomologica Hungarica, **41**, 53-67.
- Pilet, F., Pelle, R., Ellisseche, D. & Andrivon, D. 2005. Efficacy of the R2 resistance gene as a component for the durable management of potato late blight in France. Plant Pathology, **54**, 723-732.
- Rullich, G. & Schöber-Butin, B. 2001. Old and new populations of *Phytophthora infestans* in Germany. PAV-Special Report no. 7, 233-238.
- Schöber-Butin, B. 1999. *Phytophthora infestans*: pathotypes, mating types and fungicide resistance in Germany. PAV-Special Report Number 5, 178-182.
- Schöber, R. & Turkensteen, L.J. 1992. Recent and future developments in potato fungal pathology. Netherlands Journal of Plant Pathology, **98** (2), 73-83.
- Shattock, R.C., 2002. *Phytophthora infestans*: populations, pathogenicity and phenylamides. Pest Management Science, **58**, 944-950.
- Shattock, R.C., Tooley, P.W. & Fry, W.E. 1986. Genetics of *Phytophthora infestans*: Determination of recombination, segregation, and selfing by isozyme analysis. Phytopathology, **76**, 410-413.
- Sheldon, A.L. 1969. Equitability indices: Dependence on the species count. Ecology, **50**, 466-467.
- Śliwka, J., Sobkowiak, S., Lebecka, R., Avendao Córcoles, J. & Zimnoch-Guzowska, E. 2006. Mating type, virulence, aggressiveness and metalaxyl resistance of isolates of *Phytophthora infestans* in Poland. Potato Research, **49** (3), 155-166.
- Smart, C. D. & Fry, W. E. 2001. Invasions by the late blight pathogen: renewed sex and enhanced fitness. Biological Invasions **3**, 235-243.
- Spielman, L.J., Drenth, A., Davidse, L.C., Sujkowski, L.J., Gu, W., Tooley, P.W. & Fry W.E. 1991. A second world-wide migration and population displacement of *Phytophthora infestans*? Plant Pathology, **40**, 422-430.

- Sujkowski, L.S., Goodwin, S.B. & Fry, W.E. 1996. Changes in specific virulence in Polish populations of *Phytophthora infestans*: 1985-91. *European Journal of Plant Pathology*, **102**, 555-561.
- Sujkowski, L.S., Goodwin, S.B., Dyer, A.T. & Fry, W.E. 1994. Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. *Phytopathology*, **84**, 201-207.
- Tooley, P.W., Fry, W.E. & Villareal M.J. 1985. Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. *Journal of Heredity*, **76**, 431-435.
- Turkensteen, L.J., Flier, W.G., Wanningen, R. & Mulder A., 2000. Production, survival and infectivity of oospores of *Phytophthora infestans*. *Plant Pathology*, **49**, 688-696.
- Vedenyapina, E. G., Zoteyeva, N. M. & Patrikeeva, M. V. 2002. *Phytophthora infestans* in Leningrad region: virulence genes, compatibility types and oospore viability. In the USSR. *Mikologija i fitopatologija*, **36** (6), 77-85.
- Vorobyeva, Yu.V., Gridnev, V.V., Bashaeva, E.G., Pospelova, L.A., Kvasnyuk, N.Ya., Kuznetsova, L.N., Shemyakina, V.P., Morozova, E.V., Zherebtsova, L.N. & Rozalyeva, V.V. 1991. On the occurrence of the A2 mating type isolates of *Phytophthora infestans* (Mont.) d by. In the USSR. *Mikologija i fitopatologija*, pp.62-67.
- Zimnoch-Guzowska, E. 1999. Late blight and blight research in Central and Eastern Europe. In: Crissman, L., C. Lizárraga (eds). *Proceedings of the Global Initiative on Late Blight Conference: A Threat to Global Food Security*, Vol. 1, pp. 9-14., Quito, Ecuador.
- Zwankhuizen, M.J., Govers, F. & Zadoks J.C. 2000. Inoculum sources and genotypic diversity of *Phytophthora infestans* in Southern Flevoland, the Netherlands. *European Journal of Plant Pathology*, **106**, 667-680.

SUMMARY IN ESTONIAN

Kartuli-lehemädaniku tekitaja *Phytophthora infestans* Eesti populatsioonide fenotüübiline ja genotüübiline iseloomustamine

Kartuli-lehemädanik, mida põhjustab patogeen *Phytophthora infestans*, on üks kõige enam kahju tekitav kartulahaigus juba üle pooleteise sajandi nii Eestis kui mujal Euroopas. Soodsates kasvu- ja arengutingimustes võib patogeen hävitada kogu kartuli maapealse osa ja põhjustada seeläbi märkimisväärse saagikao.

Phytophthora infestans on heterotalne organism, mis vajab suguliseks paljunemiseks ja oospooride (paksukestalistes püsieosedes) moodustamiseks kahte paarumistüüpi (nimetatud A1 ja A2). Viimase kolmekümne aastaga on patogeen teinud Euroopas läbi suured muutused (Fry *et al.*, 1993). Varem esinenud 'vana' populatsioon asendus 'uue' populatsiooniga, mis oli ilmselt sisse toodud seoses mugulate impordiga Mehhikost Euroopasse 1970-ndate lõpus (Fry *et al.*, 1993). Vanal populatsioonil oli vaid üks paarumistüüp A1 ja üks mitokondrilise DNA haplotüüp Ib, kuid uues populatsioonis esinesid mõlemad paarumistüübid A1 ja A2 ning mtDNA haplotüübid Ia ja IIa. Teateid sugulisest paljunemisest on avaldatud enamikes Euroopa maades, sealhulgas Hollandis (Zwankhuizen *et al.*, 2000), Soomes, Rootsis, Norras, Taanis (Lehtinen *et al.*, 2008), Poolas (Śliwka *et al.*, 2006), Saksamaal (Schöber-Butin, 1999) ja Venemaal (Bagirova & Dyakov, 1998).

Varem paljunes seen vaid sugutul teel sporangiumide abil ja kandus edasi tuulehoogude ja vihmapiiskadega ning talvitus nakatunud seemnemugulates, säilides ladudes või pärast koristust mullas umbrohtkartulitena. Vastaspaarumistüüpide seeneniitide koostoimel tekivad antiididid ja oogoonid, mis võib viia oospooride moodustumiseni, mistõttu patogeen on võimeline paljunema suguliselt. Oospoorid võivad mullas eluvõimelisena säilida vähemalt kolm aastat (Turkensteen *et al.*, 2000) ning nad on vastupidavad ebasoodsate ilmastiku- ja keskkonnatingimuste suhtes. Oospooridest alguse saav lehemädanik võib lööbida senisest tunduvalt varem (mais, juunis) (Drenth *et al.*, 1993) ja põhjustada seeläbi suurt kartulisaagi langust ja sellest tulenevat majanduslikku kahju.

Enne käesolevat uurimustööd oli Eestis *P. infestans* geneetiliste uurimuste kohta väga vähe teada, ehkki Üleliidulise Taimepatoloogia Instituudi

teadlased tegid pidevalt sellekohast uurimistööd, kuid tulemusi ei publitseeritud, mistõttu meil puuduvad andmed selle ajajärgu kohta. Aastatel 1966-1989 määrati Eestis isoleeritud *P. infestans* tüvede rassiline koosseis (Koppel, 1996). Siiski, on olemas andmed Eestist 1983. aastal isoleeritud kahe isolaadi kohta sõrmejärgede, paarumistüübi ja allosüümide osas, mida on publitseerinud Vorobyeva *et al.* (1991), Goodwin *et al.* (1994) ja Sujkowski *et al.* (1994).

Käesoleva doktoritöö eesmärgiks on iseloomustada lehemädaniku tekitajat *P. infestans* erinevate fenoo- ja genotüübiliste iseloomustajatega (I, II, III, IV, V). Samuti otsisin vastuseid järgnevatele küsimustele: 1) kas paarumistüüpide esinemise suhte Eestis viitab sugulisele paljunemisele (I, II, III, IV) ning kas leidub viiteid oosporidest pärinevale nakkusele (II), 2) milline on fenoo- ja genotüübiline varieerumine ning kas see on võrreldes teiste Euroopa riikidega samal tasemel (I, II, III, V), 3) kas erinevate populatsiooniparameetrite osas on ajast ja piirkonnast tingitud mõjusid (I, II, III), 4) kui efektiivne on metalaksüülil põhineva fungitsiidi efektiivsus lehemädaniku tõrjes (V), 5) kas mahepõldudel on mitmekesisemad ja resistentsemad populatsioonid kui tavatootmispõldudel (IV)?

P. infestans Eesti populatsiooni iseloomustamiseks koguti kahe aasta vältel (2002-2003) 101 isolaati seitsmelt põllult (Joonis 1, I; V). Kolm proovikogumiskohta asusid tavatootmispõldudel: Ingliste 2002, Ingliste 2003 ja Kehtna 2003 Kesk-Eestist (Tabel 1; I). Need tavatootmistalud on Eestis ühed tähtsaimad seemne- ja toidukartuli kasvatajad. Kasvatatakse vaid kõrgelt sertifitseeritud seemet ja lehemädaniku tõrjeks tehakse 5-7 tõrjekorda (sõltuvalt aastast). Kaks proovikogumiskohta asusid Jõgeva Sordiaretuse Instituudi katsepõldudel: Jõgeva 2002 ja Jõgeva 2003. Seda asukohta iseloomustab peremeestaime kõrge geneetiline varieeruvus, lisaks mitmed rassispetsiifiliste geenide genotüübid. Lehemädaniku keemilist tõrjet ei teostatud. Proove koguti ka kahe väikekasvataja põldudelt Kesk-Eestist (Käru 2003) ja Kagu-Eestist (Võnnu 2003). Neis väiketootmistaludes ei kasutata sertifitseeritud seemet, mis on viirushaige, samuti puudub neil kindel külvikorra plaan. Ka lehemädaniku tõrje on väga kaootiline, varieerudes mittetõrjumisest kuni väga sagedase tõrjumiseni. Eesti populatsiooni detailsemaks kirjeldamiseks koguti aastatel 2004-2007 432 isolaati (II, V) 25 põllult (suurtootmis-, väiketootmis- ja katsepõllud) Edela-, Ida-, Kagu-, Kesk-, Lõuna- ja Põhja-Eestist (Tabel 1, II). Uurimaks võimalikke erinevusi viljelusviiside vahel (mahe-, su-

urtootmis-, väiketootmispõllud), koguti aastatel 2004-2005 196 *P. infestans* isolaati kaheteistkümnelt Põhja-Eesti põllult (Tabel 1, IV). Uurimaks ajalisi muutusi populatsiooni parameetrites koguti seitsme aasta jooksul 133 isolaati. Kõik põllud asusid Jõgeva Sordiaretuse Instituudi katsepõldudel. Keemilist lehemädaniku tõrjet ei tehtud. Enamus isolaate saadi lehtedelt, vaid väike kogus pärines mugulatest.

Kõik kogutud isolaadid viidi iseloomustamiseks puhaskultuuri (I, II, III, IV, V). Isoleerimiseks asetati nakatunud lehekoe tükk piirituses ja leegis steriliseeritud mugulalõikude vahele (I, II, III, IV, V). Kasutati vaid vastuvõtlikke R-geene mittesisaldavate sortide mugulaid (Berber 2002, 2004-2007; Bintje 2003). Lõigud asetati steriilsetesse Petri tassidesse. Petri tasse hoiti kasvukambris 16° juures 6-7 päeva, kuni mütseel oli kasvanud läbi kartulilõigu. Väike kogus mütseeli kanti mugulalõikudelt steriilse nõela abil rukki söötmele (Caten & Jinks, 1968). Puhaskultuure säilitati 5° C juures ja ümberkülve tehti iga kahe kuu järel. Kõik fenotüübilised katsed tehti isoleerimisaastal oktoobrist kuni novembrini (I, II, III, IV, V). Genotüübilised analüüsid tehti 2004. aastal maist juulini (I, III) ja 2005. aastal novembrist detsembrini (II, III).

Lehemädanikutekitaja tüved isoleeriti ühe tüüpilise haigustekitaja laiguga lehtedelt (I, II, III, IV, V). Lehemädanikust nakatunud lehed (üks taime kohta) koguti esimeste haigussümptomite ilmnemisel (I, II, IV), kasvuperioodi lõpul (IV) või kogu kasvuperioodi jooksul (III, V).

Metalaksüüliresistentsuse määramisel kasutati kõigil isolaatidel 'ujuva lehe meetodit', nagu kasutasid Hermansen *et al.* (2000), ja modifitseeriti autori poolt (I, II, IV, V). Vastuvõtliku kartulisordi (Berber 2002; Bintje 2003) lehti hoiti 3-4 minutit erineva kontsentratsiooniga (0, 10 ja 100 mg L⁻¹) metalaksüüli lahuses. Iga leht nakatati ühe tilga (20 mL⁻¹) 7-9 päeva vanusest mütseelist valmistatud sporangiumide suspensiooniga. Pärast nakatamist kaeti lehed niiskuse hoidmiseks kilega. Katset korrati kaks korda ja igas katses oli kaks kordust. Igas metalaksüüli kontsentratsiooni kombinatsioonis kasutati nelja lehte. Sporulatsiooni esinemist ja selle ulatust hinnati seitse päeva pärast nakatamist, inkubeerides lehti 15°C ja >90% relatiivse niiskuse juures. Sporulatsiooni hinnati visuaalselt järgmise skaala alusel: 0, sümptomid puuduvad; 1, väiksed nekrootilised laigud; 2, <10% nekrootiline laik; 3, 10-50% lehepinnast on kaetud mütseeliga; 4, 50-75% lehepinnast on kaetud mütseeliga; 5, >75% lehepinnast on mütseeliga kaetud. Sporulatsioon hinnati sobi-

vaks, kui kumulatiivne skoor kõigi nelja lehe puhul oli vähemalt 12. Kui 100 mg L⁻¹ kontsentratsiooni juures tekkis lehel sporulatsioon, hinnati isolaadid resistentseteks. Kui soprulatsioon tekkis metalaksüüli 10 mg L⁻¹ kontsentratsiooni puhul, hinnati need keskmise tundlikkusega isolaatideks ja isolaadid, millised sporuleerusid vees, hinnati tundlikeks (I, II, IV, V).

Kogutud isolaatide spetsiifiline virulentsus määrati resistentusgene R1-R11 sisaldavate diferentsiaatorsortide lehtede nakatamisega (I, II, IV) (Malcolmson & Black, 1966) (saadud oti Põllumajandusteaduste agentuurist). Nakatamiseks kasutati kasvuhoones või kasvukambris ettekasvatatud kuue kuni kaheksa nädala vanuste taimede täielikult väljaarenenud lehti. Lehed asetati niiskele filterpaberile lehe alaküljega ülespoole ja nakatati kahe tilga (20 mikrolitrit) haigustekitaja 7-9 päeva vanusest kultuurist valmistatud koniide ja zoospoore sisaldava suspensiooniga (1.0-4.0 x 10⁴ sporangia ml⁻¹). Nakatusjärgselt kaeti lehed niiskuse säilitamiseks polüetüleenkilega ja inkubeeriti seitse päeva 16° C juures. Nakatumine loeti sobivaks, kui soprulatsioon leiti vähemalt neljal lehel kuuest ja keskmine skoor oli vähemalt 15. Sporulatsiooni hindamiseks kasutati sama skaalat nagu metalaksüüliresistentsuse puhul.

Paarumistüüpide tuvastamiseks kasvatati *P. infestans* tüvesid Petri tassides rukki söötmel. Rukki söötmel kasvanud isolaadist lõigati välja ca 0.5*3 cm suurused korralikult väljaarenenud mütseeliga kaetud agariblokid, mis asetati Petri tassi steriilsele agarile. Ühte tassi asetati paari cm vahega uuritav isolaat ja teine juba teadaoleva paarumistüübiga testerisolaat. Testerisolaadid saadi Cornelli Ülikoolist (USA) ja Soome Põllumajaduslikust Uurimiskeskusest (MTT). Kümne kuni kaheksateistkümne päevase inkubeerimise järel 16° C juures hinnati oospooride esinemust agariblokkidelt kokkukasvanud mütseelide esinemiskohas. Oospoorid moodustuvad vaid erinevatesse paarumistüüpidesse kuuluvate isolaatide kokkupuutel, seetõttu loeti kõik A1 testerisolaadiga kokkukasvanud oospoore moodustanud tüved A2 paarumistüüpi kuuluvateks ja A2 testerisolaadiga kokkukasvamisel oospoore moodustunud tüved A1 paarumistüüpi kuuluvateks (I, II, III, IV). Mõlema testerisolaadiga oospoore moodustavad isolaadid loeti iseiljastuvateks A1A2 paarumistüüpi kuuluvateks.

RFLP sõrmejälgede määramiseks proov RG57-ga kasutati Goodwin *et al.*, (1992) poolt välja töötatud meetodikat, nagu kirjeldasid Runno-

Paurson *et al.* (2009). Mitokondrilise DNA (mtDNA) haplotüüpide määramiseks kasutati Griffith & Shaw (1998) poolt välja töötatud polümeraasi ahela reaktsioonipiiratud fragmendi pikkuse polümorfismi (PCR-RFLP) modifitseeritud meetodikat, nagu kirjeldasid Runno-Paurson *et al.*, (2009, I; 2010, II).

Metalak süüliresistentsuuringuid tehti kuue aasta vältel (2002-2007). Varasem uuring (I, V) näitas, et aastatel 2002-2003 oli uuritavate tüvede hulgas 30% metalak süüliresistentseid, 51% keskmiselt tundlikke ja 19% tundlikke isolaate (Tabel 2, I). Erinevusi kahe aasta vahel ei leitud ($\chi^2 = 2.43$, $df = 1$, $p = 0.1192$). Kõikidest proovikogumiskohtadest leiti metalak süüli suhtes tundetuid isolaate (tundlikud puuduvad). Näiteks Inglitest ja Kärust 2003. aastal kogutud proovid koosnesid vaid resistentsetest ja keskmiselt tundlikest isolaatidest (Tabel 3, I). Asukohtade vahel märkimisväärseid erinevusi ei leitud ($\chi^2 = 1.31$, $df = 4$, $p = 0.86$). Metalak süüliresistentsed isolaadid jagunesid kahe paarumistüübi vahel, kusjuures A1 paarumistüüpi oli 69% isolaatidest ja A2 paarumistüüpi 31% isolaatidest. Metalak süüliresistentsuse tase ei sõltunud märkimisväärselt paarumistüübist ($\chi^2 = 2.08$, $df = 1$, $p = 0.15$).

Hilisemal perioodil (2004-2007) kogutud isolaatide hulgas (II, V) oli metalak süüliresistentseid 37%, keskmiselt tundlikke 25% ja tundlikke 37%. Metalak süüliresistentsete isolaatide proportsioon varieerus märkimisväärselt erinevate asukohtade ($\chi^2 = 42.20$, $df = 11$, $p < 0.001$) ja aastate ($\chi^2 = 61.57$, $df = 3$, $p < 0.001$) vahel. Varieeruvus oli sõltuvalt asukohast 0-81% (Joonis 1, V). Metalak süüliresistentsete isolaatide määr oli kõrge 2004. aastal (56%), samas 2007. aastal olid enamused uuritud isolaate tundlikud metalak süüli suhtes (Tabel 2, II). Vaid kolmes asukohas 25-st ei leitud metalak süüliresistentseid isolaate. Märkimisväärsed erinevusi põllutüüpide vahel (suurtootmis-, väiketootmis- ja katsepõllud) ei leitud ($\chi^2 = 0.05$, $df = 1$, $p = 0.82$) (II). Metalak süüliresistentsed isolaadid olid ülekaalus katsepõldudel 2004. aastal (67%) ja tootmispõldudel 2004. ja 2005. aastal (60%) kogutud isolaatide hulgas (V). Leiti tugev seos metalak süüliresistentsuse ja põldude vahel, kus metalak süüli sisaldavaid fungitsiide kasutati ($\chi^2 = 9.24$, $df = 1$, $p = 0.0024$). Põldudel, kus lehemädanikku oli tõrjutud metalak süüli sisaldava preparaadiga, olid ligi pooled (48%) isolaatidest resistentsed, samas kui põldudel, kus tõrjet ei tehtud metalak süüli toimeainet sisaldava preparaadiga, oli resistentseid isolaate vaid 33% (Tabel 3, II). Ei leitud märkimisväärselt seost metalak süüliresistentsuse ja paarumistüübi vahel ($\chi^2 = 1.99$, $df = 2$, $p = 0.36$).

Kõik teadaolevad virulentsusfaktorid (R1-R11) leiti mõlemast andmekogust (I, II). Mõlemas uurimustöös (I, II) oli virulentsus differentsiaatorsortide R1, R3, R4, R7, R10 ja R11 suhtes sage. Virulentsus differentsiaatorsortide R5 ($5\% \pm 2.4$ SE) ja R9 ($14\% \pm 7.2$ SE) suhtes oli suhteliselt madal (Joonis 2, I) aastatel 2002-2003. Aastatel 2004-2007 kogutud isolaatide hulgas oli virulentsussagedus samuti madal ($F_{(10,66)} = 15.89$, $p < 0.0001$) diferentsiaatorsortide R9 ja R5 puhul (Joonis 1, II). Enamus isolaate (90-96%) olid võimelised allutama/nakatama neli R geeni (Tabel 4, I, Tabel 4, II).

Patotüüp 1.3.4.7.10.11 oli kõige enam levinud mõlemal ajaperioodil (I, II) (Tabel 4, I; Tabel 4, II). Ajavahemikul 2002-2003 (I) kogutud 101 isolaadi hulgas leiti 66 patotüüpi (Tabel 4, I) ja aastatel 2004-2007 (II) kogutud 432 isolaadi hulgas vaid 87 patotüüpi (Tabel 4, II). Kui varasemas kollektsioonis (I) moodustas kolm enamlevinud patotüüpi vaid 17% uuritud isolaatidest, siis hilisemas kollektsioonis moodustas neli enamlevinud patotüüpi peaaegu poole (46%, II) kõigist isolaatidest. Aastatel 2002-2003 kogutud isolaatide hulgas oli väga kõrge (>49%) unikaalsete (leiti vaid korra) patotüüpide osakaal (Tabel 4, I), kuid hilisemal perioodil (II) kogutud isolaatide hulgas oli unikaalsete patotüüpide osakaal peaaegu 4 korda (13%) madalam (Tabel 4, II). Virulentsuse keskmine arv isolaadi kohta oli varasemas uuringus 6.3 (I) ja hilisemas uuringus 6.9 (II). Varieeruvus erinevate põldude vahel oli väga suur, varieerudes väga madala virulentsusega (4.3) 2003. aastal Võnnust kogutud isolaatidest (Tabel 5, I) kuni väga kõrge (8.9) virulentsuseni Jõgeval 2005. aastal kogutud isolaatidest (Tabel 5, II). Normaliseeritud Shannon'i indeks oli 2002-2003 (I) väga kõrge (0.92), võrreldes 2004-2007 (II) kogutud isolaatidega (0.54). Märkimisväärseid erinevusi täheldati spetsiifilises virulentsuses erinevate aastate (II, $F_8 = 9.41$, $p < 0.001$) ja askohtade (I, II) vahel ($F_{4,10} = 3.79$, $p = 0.017$). Erinevusi põllutüüpide vahel ei leitud (II).

Varasemal perioodil (I) (2002-2003) kogutud isolaatide hulgas oli 60% A1 paarumistüüpi ja 40% A2 paarumistüüpi (Tabel 2, I). Mõlemad paarumistüübid eksisteerisid koos kolmel põllul seitsmest (Tabel 2, I). 2003. Leiti märkimisväärne seos ($\chi^2 = 18.54$, $df = 4$, $p < 0.001$) A1 ja A2 paarumistüüpide proportsioonis. Hilisemas kollektsioonis (II) olid A1 paarumistüüpi 64% isolaatidest, A2 paarumistüüpi 33% isolaatidest ja vaid 2% oli iseviljuvaid. Mõlemad paarumistüübid eksisteerisid koos kõigil 25-l uuritud põllul. A2 paarumistüübi sagedus varieerus asukohati 3-71% piires. A2 paarumistüübi tase oli väga kõrge 2004. ja 2007. aastal,

kus peaaegu pool (vastavalt 44% ja 48%) uuritud isolaatidest kuulusid A2 paarumistüüpi. Leiti märkimisväärne erinevus kahe paarumistüübi esinemuses asukohtade ($\chi^2 = 28.68$, $df = 10$, $p = 0.0014$) ja aastate ($\chi^2 = 26.28$, $df = 3$, $p < 0.001$) vahel. Väiketootjate põldudelt kogutud isolaatide hulgas oli A2 paarumistüübi sagedus kõrgem (39%) kui suurtootjate põldudelt kogutud isolaatide hulgas (26%) ($\chi^2 = 7.05$, $df = 1$, $p = 0.0079$).

Pikaajaline uuring (III) ühes asukohas (Jõgeval) näitas, et ehkki A1 ja A2 paarumistüübi sagedused (vastavalt 59% ja 38%) olid sarnased varem leitutele (I, II, IV), leiti siiski märkimisväärsed erinevused ($\chi^2 = 45.74$, $df = 12$, $p < 0.001$) paarumistüüpide proportsioonis uuritud aastatel vahel (III). A2 paarumistüübi sagedus oli üsna kõrge aastatel 2001, 2003, 2004, 2005 ja 2007 (41-71%) (Joonis 1, III). Kuna aga A2 paarumistüüpi ei leitud 2002. aastal (Joonis 1, III) ja A2 paarumistüübi sagedus oli väga madal 2006. aastal (3%), siis A2 paarumistüübi keskmine sagedus langes.

DNA sõrmejälgede määramisel proov RG57-ga leiti kahekümne viiest võimalikust fragmendist 17 fragmenti. Kaheksat fragmenti (4, 11, 12, 15, 17, 19, 22, 23) ei leitud üldse ja neli (13, 14, 24, 25) esines kõigil isolaatidel (Tabel 2, I; Tabel 6, II). Ülejäänud 13 fragmenti (1, 2, 3, 5, 6, 7, 8, 9, 10, 16, 18, 20, 21) olid polümorfseid. Kokku leiti 38 proov RG57 sõrmejälge. Neist viis levinuimat sõrmejälge (I, II, III, XX and XXII) (Tabel 2, I; Tabel 6, II) moodustas üle poole (57%) isolaatidest. Leiti 25 unikaalset sõrmejälge kolmeteistkümnest asukohast, kusjuures enamis isolaate oli kogutud 2003. ja 2005. aastal (Tabel 2, I; Tabel 6, II).

Aastatel 2002-2003 kogutud isolaatide hulgast leiti neljast teadaolevast mitokondrilise DNA haplotüübist kaks, Ia ja IIa (I). Kaksikümmend kolm isolaati (46%) olid Ia haplotüüpi ja 27 isolaati (54%) olid IIa haplotüüpi. Need haplotüübid esinesid nii A1 kui A2 paarumistüüpi isolaatidel (Tabel 2, I). Hilisemal perioodil (II) kogutud isolaatidel leiti kolm haplotüüpi (Ia, IIa, IIb; II). Uuritud 57 isolaadi hulgas oli 30 isolaati Ia haplotüüpi (51%), 24 isolaati IIa haplotüüpi (42%) ja 3 isolaati IIb haplotüüpi (7%). Kolmekümne seitsme A1 paarumistüüpi isolaadi hulgas oli 20 isolaati Ia haplotüüpi, 14 isolaati IIa ja 3 isolaati IIb haplotüüpi (Tabel 6, II). Kahekümne A2 paarumistüüpi isolaadil oli jaotuvus järgmine: 10 isolaati Ia ja 10 isolaati IIa (Tabel 6, II). Ia ja IIa hap-

lotüüpide sagedustes esinesid asukohtade vahel erinevused ($\chi^2 = 40.95$, $df = 26$, $p = 0.031$).

Aastatel 2002-2005 kogutud isolaatidel indentifitseeriti 55 multilookus-genotüüpi, millest 45 (86%) leiti vaid ühel korral. Enamus neist unikaalsetest genotüüpidest pärines Jõgevalt (2002-2003) (Tabel 2, I) ja 2005. aastal (Tabel 6, II) kogutud isolaatide hulgast. Kõik multilookus-genotüübid kuulusid 'uude' populatsiooni, mis asendas US-1 kloonilise põlvkonna (A1 paarumistüüp, Ib mtDNA haplotüüp), nagu kirjeldasid Spielman *et al.* (1991) ja Lebreton & Andrivon (1998). Neli enamlevinud genotüüpi oli EE-2 (13 isolaati), EE-1 (8 isolaati), EE-7 (6 isolaati) ja EE-4 (5 isolaati). Ainult kolm multilookus genotüüpi leiti kõigil neljal aastal (Tabel 2, I; Tabel 6, II).

Genotüübilist mitmekesisust mõõdeti normaliseeritud Shannon'i indeksiga, mis osutus kõrgeks (0.76, I; 0.79, II). Enamus RG57 sõrmejälgi ei asotsieerunud ühegi mtDNA haplotüübiga (Tabel 6, II). Metalaktsüüliresistentsuse ja mitokondrilise DNA haplotüüpide vahel seost ei leitud ($\chi^2 = 1.66$, $df = 3$, $p = 0.65$) (II). Ka ei leitud seost proov RG57 sõrmejälgedel (I, II), metalaktsüüliresistentsuse või paarumistüüpide vahel (I, II, IV).

Viljelusviiside võrdlusuuringus (IV) leiti märkimiväärseid erinevusi metalaktsüüliresistentsuses erinevate viljelusviiside vahel ($\chi^2 = 23.75$, $d.f. = 2$, $p < 0.0001$). Suurtootmispõldudel olid kuni 66% analüüsitud isolaatidest resistentsed metalaktsüüli suhtes, samas kui väiketootjate põldudel oli sama näit vaid 26%. Veelgi madalam oli see mahepõldudel, kus metalaktsüüliresistentsid tüvesid oli vaid 14% (Tabel 3, IV). Puudusid erinevused isolaatide kogumisaastate vahel ($\chi^2 = 0.98$, $d.f. = 1$, $p = 0.42$). Seos metalaktsüüliresistentsuse ja paarumistüübi vahel ei olnud märkimisväärne ($\chi^2 = 3$, $d.f. = 1$, $p = 0.083$).

Viljelusviiside uuringus (IV) olid peaaegu kõik isolaadid virulentsed diferentsiaator-genotüüpide R1, R3, R4, R7, R10 and R11 suhtes. Virulentsusfaktor 9 (1%) oli väga haruldane ning faktorid 5 (10%) ja 8 (10%) esinesid suhteliselt harva (Joonis 1, Tabel 4, IV). Leiti 38 patotüüpi (Tabel 5, IV), kusjuures kaks kõige enam levinud patotüüpi moodustasid enamuse (70%) analüüsitud isolaatidest (Tabel 5, IV). Virulentsuskompleks oli kõrgeim mahepõldudel (7.3). Kompleksed patotüübid domineerisid mahepõldudel ja olid vähem levinud väike- ja suurtootjate põldudel ($F_{(193)} = 8.49$, $p = 0.00029$). Normaliseeritud Shannon'i indeks erines

märkimisväärselt erinevate viljelusviiside vahel ($F_{(2)} = 23.89$, $p = 0.0028$). Suurtootjate põldudel oli indeks väga kõrge (0.71), väiketootjate (0.13) ja mahepõldudel tunduvalt madalam (0.18).

A2 paarumistüübi esinemissagedus oli kõrgeim mahepõldudel ja madalaim suurtootjate põldudel (Tabel 2, IV) ($\chi^2 = 9.60$, d.f. = 2, $p = 0.0082$). Mõlemad paarumistüübid koos ühel põllul leiti 11-l põllul 12-st. A2 paarumistüübi proportsioon tõusis järsult 28%-lt 2004. aastal kuni 54%-ni 2005. aastal ($\chi^2 = 11.87$, d.f. = 1, $p = 0.0006$).

Suurtootjate põldudelt leiti kaks Euroopas haruldase haplotüübiga (IIb) isolaati. Viljelusviiside vahel leiti märkimisväärsed erinevused haplotüüpide osas ($\chi^2 = 8.38$, d.f. = 2, $p = 0.015$), kus IIa haplotüübi sagedus oli kõrgeim suurtootjate põldudel ja madalaim mahepõldudel, kust leiti vaid Ia haplotüüpi isolaate (Tabel 6, IV). Genotüübiline mitmekesisus varieerus märkimisväärselt viljelusviiside vahel ($F_{(2)} = 41.76$, $p < 0.001$). Suurtootjate põldudel oli see indeks ülikõrge (0.97), väiketootjate põldudel (0.53) ja mahepõldudel tunduvalt madalam (0.28).

Minu uurimustöö kinnitab Goodwin'i *et al.* (1994) ja Vorobyeva *et al.* (1991) poolt esitatud varasemaid teateid, mille kohaselt *P. infestans* Eesti populatsioon aastatel 2002-2003 (I) ja 2004-2007 (II) on üldjoontes sarnane Euroopa populatsiooni iseloomustajatega. Alates 1980. aastate algusest on tulnud pidevalt teateid dramaatilistest muutustest *P. infestans* Euroopa populatsioonides, kus on kõrge patotüübiline mitmekesisus, esinevad mõlemad paarumistüübid ja metalaksüüliresistentsus. Selliseid muutusi on täheldatud paljudes Euroopa riikides (Spielman *et al.*, 1991; Drenth *et al.*, 1994; Sujkowski *et al.*, 1994; Sujkowski *et al.*, 1996; Brurberg *et al.*, 1999; Hermansen *et al.*, 2000; Bakonyi *et al.*, 2002a; Day *et al.*, 2004; Nagy *et al.*, 2006; Śliwka *et al.*, 2006; Lehtinen *et al.*, 2008; Lees *et al.*, 2009). Minu uurimustöö näitab, et *P. infestans* Eesti populatsioon on kõrge patotüübilise mitmekesisusega, esinevad mõlemad paarumistüübid, mis eksisteerivad enamikel põldudel koos. Väga levinud on ka metalaksüüliresistentsus, mis kinnitab eelnevaid arvamusi varem tehtud uurimustöodes.

Minu uurimustööde (I, II) tulemustest selgus, et *P. infestans* Eesti populatsioonid on mitmekesised, omades suurel arvul multilookusgenotüüpe, millest enamus on unikaalsed ja omased vaid eesti populatsioonile (I, II).

Võrreldes minu doktoritöö tulemusi (I, II, III, IV) mujal Euroopas tehtud töödega võib öelda, et Eesti *P. infestans* populatsioon on silmnähtavalt sarnane Venemaa ja Põhjamaade populatsioonidega. Ehkki Eesti kasvatab enamuse seemnekartulist ise ja impordib vaid 5% seemnekartulit Hollandist, on selge, et toimub piisav geenivoog, mistõttu Eesti populatsioon on sarnane naabermaade populatsioonidega (I, II, III, IV).

Meetalaksüüliresistentsuse märkimisväärne ajaline kõikumine uuritud aastate vältel (2002-2007) *P. infestans* populatsioonis on ilmselt seotud intensiivsema metalaksüüli põhineva fungitsiidi kasutamisega. Meie seisukoht on, et nii kaua kuni metalaksüüli baseeruvaid fungitsiide kasutatakse vastavalt ettenähtud soovitudele, on vähe tõenäoline, et metalaksüülitundlikud tüved asenduvad Eestis tundlike tüvedega (V).

Uurimustöö tulemused näitasid, et *P. infestans* Eesti populatsioon on mitmekesine ning ilmselgelt toimub siin suguline paljunemine, sest A2 paarumistüübi esinemise määr on kõrge ja mõlemad paarumistüübid esinevad enamasti koos ühel põllul (I, II, III, IV). Siiski on vaja rohkem informatsiooni, et selgitada oospooride rolli Eesti lehemädanikutekitaja epidemioloogias (II).

Viljelusviiside uurimuse tulemused (IV) näitasid, et erinevate viljelusviiside vahel võivad olla märkimisväärsed erinevused erinevates aspektides *P. infestans* Eesti populatsiooni struktuuris. Erinevused ilmnisid paarumistüüpide, virulentsusegeenide, mtDNA haplotüüpide ja metalaksüüliresistentsuse osas. Tulemused ei olnud alati sarnased eelnevate uurimustööde omadega (I, II), pakkudes märkimisväärselt ajast ja kohast tingitud erinevusi *P. infestans* populatsioonis. Mitu aspekti, nagu A2 paarumistüübi kõrgem esinemismäär, mõlema paarumistüübi koosseksisteerimine enamusel põldudest ja võimalik külvikorra reeglite eiramine lubavad oletada, et mahepõldudel on suurem risk suguliseks paljumiseks võrreldes teiste viljelusviisidega (IV).

Minu uurimustöös (III) kirjeldatud populatsiooni iseloomustajad ei olnud alati sarnased võrrelduna eelnevate uuringutega nii siin kui teistes geograafilistes regioonides, oletades märkimisväärsed piirkondlike ja ajalisi varieerumisi *P. infestans* populatsiooni parameetrites. See võib vihjata sellele, et kartulikasvatavad peaksid optimaalsete otsuste tegemisel arvesse võtma regionaalset olukorda (III).

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CHARACTERIZATION OF *PHYTOPHTHORA INFESTANS*
ISOLATES COLLECTED FROM POTATO
IN ESTONIA DURING 2002-2003.

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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*.

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Keywords Diversity · Mating type ·
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RG57 fingerprinting · Virulence phenotype

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

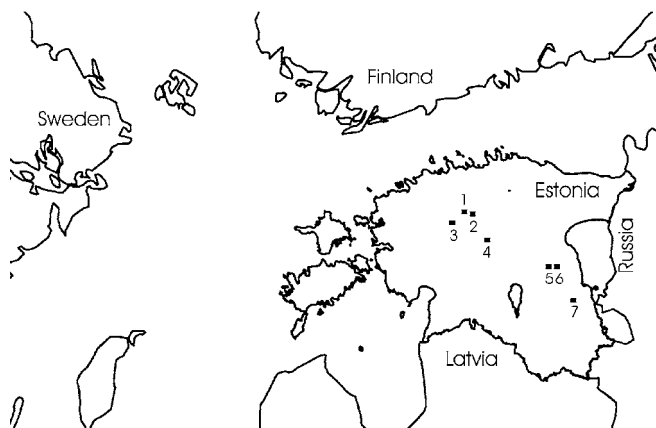
Collection and culture of isolates

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: Kärü 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 = Inglise 2002, 2 = Inglise 2003, 3 = Kehтна 2003, 4 = Kärü 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	Inglise 2002 (1)	6	2
	Inglise 2003 (2)	10	6
	Kehтна 2003 (3)	12	4
	Kärü 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	1010101001001101000110011	I	A1	Ia	I	3	2	Jõgeva 02–03
EE-2	1010101001001101000110011	I	A1	IIa	I, R,S	7	2	Ingliste 02–03
EE-3	1010101001001101000110011	I	A2	Ia	I	3	2	Kehtna 03, Võnnu 03
EE-4	1010101001001101010110011	II	A1	Ia	I, R	5	3	Jõgeva 02–03, Võnnu 03
EE-5	1010101001001101010110011	II	A1	IIa	I	1	1	Võnnu 2003
EE-6	1010101001001101010110011	II	A2	IIa	R, S	2	1	Võnnu 2003
EE-7	1110011001001100000110011	III	A1	IIa	S, I, R	6	1	Jõgeva 2002
EE-8	0010101001001100000110011	IV	A2	Ia	I, R	4	1	Käru 2003
EE-9	1000100001001101000110011	V	A2	Ia	R	1	1	Jõgeva 2003
EE-10	1000100001001101000110011	V	A2	IIa	I	1	1	Jõgeva 2003
EE-11	1110001101001101000110011	VI	A2	IIa	I	2	1	Võnnu 2003
EE-12	1110101001001101010110011	VII	A2	Ia	I	1	1	Võnnu 2003
EE-13	1110101001001101010110011	VII	A2	IIa	I	1	1	Võnnu 2003
EE-14	1110111001001101000110011	VIII	A1	IIa	S	1	1	Võnnu 2003
EE-15	1110111001001101000110011	VIII	A2	IIa	I	1	1	Võnnu 2003
EE-16	1010001001001100000110011	IX	A2	Ia	I	1	1	Jõgeva 2003
EE-17	1010011000001100000110011	X	A1	IIa	R	1	1	Ingliste 2002
EE-18	1010011001001100000110011	XI	A1	IIa	I	1	1	Kehtna 2003
EE-19	1010100000001101010110011	XII	A1	Ia	R	1	1	Jõgeva 2002
EE-20	1010101001001100000110011	XIII	A1	IIa	S	1	1	Jõgeva 2002
EE-21	1010101101001100000010011	XIV	A1	Ia	I	1	1	Jõgeva 2003
EE-22	1010101101001101000110011	XV	A2	Ia	I	1	1	Kehtna 2003
EE-23	1010101101001101010110011	XVI	A1	IIa	R	1	1	Jõgeva 2003
EE-24	1110001001001101000110011	XVII	A2	Ia	R	1	1	Käru 2003
EE-25	1110101001001100000110011	XVIII	A1	IIa	R	1	1	Jõgeva 2002
EE-26	1000100101001101010110011	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 ($1.0\text{--}4.0 \times 10^4$ 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 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 MgCl₂ (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 *HhaI* (Invitrogen) and *MspI* (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): $H_s = -\sum g_i \ln g_i / \ln N$, 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 virulence, Type III ANOVA and Tukey HSD post-hoc tests ($\alpha=0.05$) 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 Kärü 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, $df=1$, $P=0.15$).

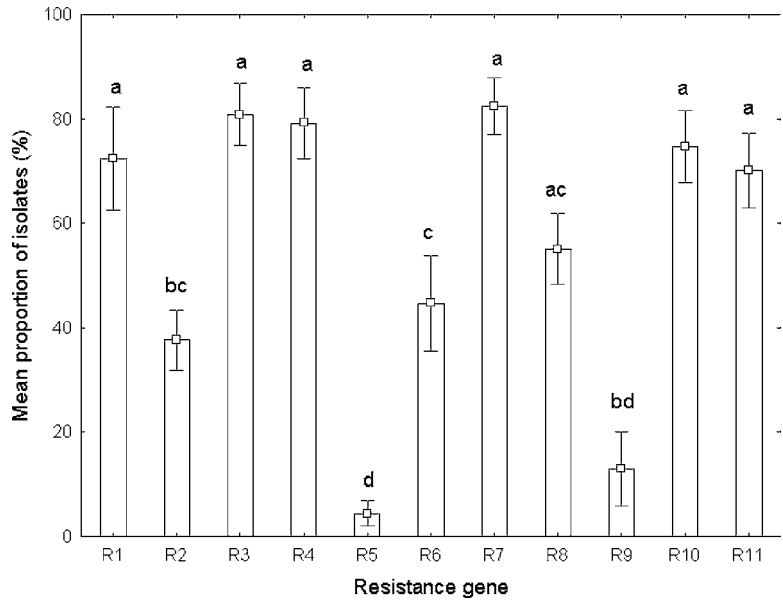
RG57 analysis

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 *Phytophthora 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

Table 4 (continued)

Race	Number of virulence factors	Number of isolates
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, Vladivostok 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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References

- Anderson, B., Sandström, M., & Strömberg, A. (1998). Indication of soil-borne inoculum of *Phytophthora infestans*. *Potato Research*, 41, 305–310. doi:10.1007/BF02358962.
- Andrivo, D. (1994). Races of *Phytophthora infestans* in France, 1991–1993. *Potato Research*, 37, 279–286. doi:10.1007/BF02360520.
- Bakonyi, J., Ládai, M., Dula, T., & Érsek, T. (2002a). Characterisation of isolates of *Phytophthora infestans* from Hungary. *European Journal of Plant Pathology*, 108, 139–146. doi:10.1023/A:1015035319685.
- Bakonyi, J., Heremans, B., & Jamart, G. (2002b). Characterization of *Phytophthora infestans* isolates collected from potato in Flanders, Belgium. *Phytopathology*, 150, 512–516. doi:10.1046/j.1439-0434.2002.00778.x.
- Brurberg, M. B., Hannukala, A., & Hermansen, A. (1999). Genetic variability of *Phytophthora infestans* in Norway and Finland as revealed by mating type and fingerprint probe RG57. *Mycological Research*, 12, 1609–1615. doi:10.1017/S0953756299008771.
- Caten, C. E., & Jinks, J. L. (1968). Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. *Canadian Journal of Botany*, 46, 329–348. doi:10.1139/b68-055.
- Cooke, L., Carlisle, D. J., Donaghy, C., Quinn, M., Perez, F. M., & Deahl, K. L. (2006). The Northern Ireland *Phytophthora infestans* population 1998–2002 characterized by genotypic and phenotypic markers. *Plant Pathology*, 55, 320–330. doi:10.1111/j.1365-3059.2006.01335.x.
- Day, J. P., & Shattock, R. C. (1997). Aggressiveness and other factors relating to displacement of populations of *Phytophthora infestans* in England and Wales. *European Journal of Plant Pathology*, 103, 379–391. doi:10.1023/A:1008630522139.
- Day, J. P., Wattier, R. A. M., Shaw, D. S., & Shattock, R. C. (2004). Phenotypic and genotypic diversity in *Phytophthora infestans* on potato in Great Britain, 1995–98. *Plant Pathology*, 53, 303–315. doi:10.1111/j.0032-0862.2004.01004.x.
- Drenth, A., Tas, I. C. Q., & Govers, F. (1994). DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. *European Journal of Plant Pathology*, 100, 97–107. doi:10.1007/BF01876244.
- Elansky, S., Smirnov, A., Dyakov, Y., Dolgova, A., Filippov, A., Kozlovski, B., et al. (2001). Genotypic analysis of Russian isolates of *Phytophthora infestans* from the Moscow region. *Siberia and Far East. Phytopathology*, 149, 605–611.
- Fry, W. E., Goodwin, S. B., Dyer, A. T., Matuszak, J. M., Drenth, A., Tooley, P. W., et al. (1993). Historical & recent migrations of *Phytophthora infestans*: chronology, pathways, and implications. *Plant Disease*, 77, 653–661.
- Fry, W. E., Goodwin, S. B., Matuszak, J. M., Spielman, L. J., Milgroom, M. G., & Drenth, A. (1992). Population genetics and intercontinental migrations of *Phytophthora infestans*. *Annual Review of Phytopathology*, 30, 107–129. doi:10.1146/annurev.py.30.090192.000543.
- Gisi, U., & Cohen, Y. (1996). Resistance to phenylamide fungicides: A case study with *Phytophthora infestans* involving mating type and race structure. *Annual Review of Phytopathology*, 34, 549–572. doi:10.1146/annurev.phyto.34.1.549.
- Goodwin, S. B., Drenth, A., & Fry, W. E. (1992). Cloning and genetic analyses of two highly polymorphic, moderately

- repetitive nuclear DNAs from *Phytophthora infestans*. *Current Genetics*, 22, 107–115. doi:10.1007/BF00351469.
- Goodwin, S. B., Cohen, B. A., & Fry, W. E. (1994). Planglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Sciences of the United States of America*, 91, 11591–11595. doi:10.1073/pnas.91.24.11591.
- Goodwin, S. B., Schneider, R. E., & Fry, W. E. (1995). Use of cellulose acetate electrophoresis for rapid identification of allozyme genotypes of *Phytophthora infestans*. *Plant Disease*, 79, 1181–1185.
- Goodwin, S. B., Smart, C. D., Sandrock, R. W., Deahl, K. L., Punja, Z. K., & Fry, W. E. (1998). Genetic change within populations of *Phytophthora infestans* in the United States and Canada during 1994 to 1996: Role of migration and recombination. *Phytopathology*, 88, 939–949. doi:10.1094/PHTO.1998.88.9.939.
- Griffith, G. W., & Shaw, D. S. (1998). Polymorphisms in *Phytophthora infestans*: Four mitochondrial haplotypes are detected after PCR amplification of DNA from pure cultures or from host tissue. *Applied and Environmental Microbiology*, 64, 4007–4014.
- Hannukkala, A. O., Kaukoranta, T., Lehtinen, A., & Rahkonen, A. (2007). Late-blight epidemics on potato in Finland, 1933–2002; increased and earlier occurrence of epidemics associated with climate change and lack of rotation. *Plant Pathology*, 56, 167–176. doi:10.1111/j.1365-3059.2006.01451.x.
- Hermansen, A., Hannukkala, A., Hafskjold Naerstad, R., & Brurberg, M. B. (2000). Variation in populations of *Phytophthora infestans* in Finland and Norway: mating type, metalaxyl resistance and virulence phenotype. *Plant Pathology*, 49, 11–22. doi:10.1046/j.1365-3059.2000.00426.0078.
- Knapova, G., & Gisi, U. (2002). Phenotypic and genotypic structure of *Phytophthora infestans* populations on potato and tomato in France and Switzerland. *Plant Pathology*, 51, 641–653. doi:10.1046/j.1365-3059.2002.00750.x.
- Lebreton, L., & Andrivon, D. (1998). French isolates of *Phytophthora infestans* from potato and tomato differ in phenotype and genotype. *European Journal of Plant Pathology*, 104, 583–594. doi:10.1023/A:1008662518345.
- Lehtinen, A., Hannukkala, A., Rantanen, T., & Jauhainen, L. (2006). Phenotypic and genetic variation in Finnish potato-late blight populations, 1997–2000. *Plant Pathology*, 56, 480–491. doi:10.1111/j.1365-3059.2006.01556.x.
- Malcolmson, J. F., & Black, W. (1966). New R genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica*, 15, 199–203. doi:10.1007/BF00022324.
- Mayton, H., Smart, C. D., Moravec, B. C., Mizubuti, E. S. G., Muldoon, A. E., & Fry, W. E. (2000). Oospore survival and pathogenicity of single oospore recombinant progeny from a cross involving US-17 and US-8 genotypes of *Phytophthora infestans*. *Plant Disease*, 84, 1190–1196. doi:10.1094/PDIS.2000.84.11.1190.
- Nagy, Z. Á., Bakonyi, J., Som, V., & Érsek, T. (2006). Genetic diversity of the population of *Phytophthora infestans* in Hungary. *Acta Phytopathologica et Entomologica Hungarica*, 41, 53–67. doi:10.1556/APHYT.41.2006.1-2.6.
- Schöber, R., & Turkensteen, L. J. (1992). Recent and future developments in potato fungal pathology. *Netherlands Journal of Plant Pathology*, 98(Suppl 2), 73–83. doi:10.1007/BF01974474.
- Sheldon, A. L. (1969). Equitability indices: Dependence on the species count. *Ecology*, 50, 466–467. doi:10.2307/1933900.
- Śliwka, J., Sobkowiak, S., Lebecka, R., Avendaño Córcoles, J., & Zimnoch-Guzowska, E. (2006). Mating type, virulence, aggressiveness and metalaxyl resistance of isolates of *Phytophthora infestans* in Poland. *Potato Research*, 49(3), 155–166.
- Spielman, L. J., Drenth, A., Davidse, L. C., Sujkowski, L. J., Gu, W., Tooley, P. W., et al. (1991). A second world-wide migration and population displacement of *Phytophthora infestans*?. *Plant Pathology*, 40, 422–430. doi:10.1111/j.1365-3059.1991.tb02400.x.
- Sujkowski, L. S., Goodwin, S. B., Dyer, A. T., & Fry, W. E. (1994). Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. *Phytopathology*, 84, 201–207. doi:10.1094/Phyto-84-201.
- Sujkowski, L. S., Goodwin, S. B., & Fry, W. E. (1996). Changes in specific virulence in Polish populations of *Phytophthora infestans*: 1985–91. *European Journal of Plant Pathology*, 102, 555–561. doi:10.1007/BF01877022.
- Turkensteen, L. J., Flier, W. G., Wanningen, R., & Mulder, A. (2000). Production, survival and infectivity of oospores of *Phytophthora infestans*. *Plant Pathology*, 49, 688–696. doi:10.1046/j.1365-3059.2000.00515.x.
- Vorobyeva, Y. V., Gridnev, V. V., Bashaeva, E. G., Pospelova, L. A., Kvasnyuk, N.Y., Kuznetsova, L. N., et al. (1991). On the occurrence of the A2 mating type isolates of *Phytophthora infestans* (Mont.) d by. in the USSR. *Mikologija i fitopatologija*, pp.62–67.
- Zimnoch-Guzowska, E. (1999). *Late blight and blight research in Central and Eastern Europe*. In: *Proceedings of the Global Initiative on Late Blight Conference*. Late Blight: A Threat to Global Food Security Vol 1, pp. 9–14.
- Zwankhuizen, M. J., Govers, F., & Zadoks, J. C. (2000). Inoculum sources and genotypic diversity of *Phytophthora infestans* in Southern Flevoland, the Netherlands. *European Journal of Plant Pathology*, 106, 667–680. doi:10.1023/A:1008756229164.



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PHENOTYPIC AND GENOTYPIC
CHARACTERISATION OF ESTONIAN ISOLATES
OF *PHYTOPHTHORA INFESTANS* IN 2004-2007

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Running title: Characterisation of Estonian isolates of *Phytophthora infestans*

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Summary

A collection of 432 single-lesion isolates of *Phytophthora infestans* collected from blighted potato foliage during 2004-2007 in Estonia, were analyzed for virulence (all isolates), mating type (424 isolates) and response to metalaxyl (412 isolates). The samples came from 25 fields comprising conventional production in central, northern, southern, south-eastern and south-western regions and from untreated experimental field trials at Jõgeva Plant Breeding Institute in eastern Estonia. Of the isolates 33% were A2 mating type. Both mating types were present in all fields; the frequency of A2 mating type varied from 3% to 71%. In the context of specific virulence, the Estonian population had a very low fre-

quency of virulence against R5 (17%) and R9 (3%). The most common pathotype was 1.3.4.7.10.11. A subgroup of 57 isolates was assessed for mtDNA haplotype and RG57 fingerprints. Three mitochondrial DNA haplotypes, i.e. Ia (51%), IIa (42%) and IIb (7%), were found. Twenty-one RG57 fingerprints were detected. The four most common fingerprints represented more than half of the isolates (67%). On the basis of combined markers, thirty-three multilocus genotypes were identified, of which 81% were detected only once. Genotypic diversity measured by the normalized Shannon diversity index was 0.79. The data indicate that the Estonian population of *P. infestans* is diverse, having a large number of multilocus genotypes and both mating types within fields, with potential for sexual recombination and spread of fungicide resistance.

Key words: mating type, metalaxyl resistance, mitochondrial DNA haplotype, *Phytophthora infestans*, RG57 fingerprinting, virulence

Introduction

Phytophthora infestans (Mont.) de Bary is one of the most serious and economically important pathogens of potato worldwide. Under favourable conditions it can destroy the whole potato haulm and cause considerable yield loss. In Estonia, the average loss due to late blight can reach 20-25% and in untreated fields even more. Without control of potato late blight is not possible to achieve high high-quality yields. In Estonia, fungicides are used routinely in conventional potato production, but under favourable conditions for the disease, with heavy pathogen pressure, it is difficult to protect large areas.

Late blight is caused by the fungus-like oomycete *P. infestans*, which can reproduce both sexually and asexually. For sexual reproduction, *P. infestans* requires both A1 and A2 mating types to produce gametangia (Fry and Goodwin, 1997). The centre of diversity of this oomycete is in the highlands of Mexico (Fry and Goodwin, 1997), where both mating types have always been present. The population is especially virulent and remarkably diverse for neutral genetic markers (Fry *et al.*, 1993). Previous studies have shown that the population in Mexico is highly sub-structured (Fry *et al.*, 1992). At least two different migration events have occurred from Mexico. The first is postulated to have occurred before 1845, after which time *P. infestans* swept through Europe and Ireland resulting in the death

of over one million people due to starvation, and emigration of 1.5 million people to other parts of Europe or North America (Drenth *et al.*, 1994). The second migration occurred in the 1970s to 1990s, bringing the A2 mating type out of Mexico and also containing genetically diverse and aggressive strains (Fry *et al.*, 1992; Fry *et al.*, 1993). Analyses of allozyme markers (Tooley *et al.*, 1985; Shattock *et al.*, 1986) and DNA fingerprints (Goodwin *et al.*, 1992a, 1992b; Drenth *et al.*, 1993) of isolates from a number of locations in the world supported the notion that the aforementioned changes resulted from displacement of an 'old' worldwide clonal lineage (US-1) by a new population (Spielman *et al.*, 1991; Fry *et al.*, 1993; Drenth *et al.*, 1994). The 'new genotypes' also proved to be generally more aggressive than the old clonal lineage (Carlisle *et al.*, 2002; Shattock, 2002). In Estonia, the A2 mating type was detected for the first time in 1987 (Vorobyeva *et al.*, 1991).

The control of potato late blight is massively dependent on the use of fungicides (Goodwin *et al.*, 1996). Resistance to metalaxyl was first recorded in *P. infestans* in Ireland and The Netherlands in 1980 (Davidse *et al.*, 1981; Dowley and O'Sullivan, 1981). Although the old population was largely sensitive to phenylamides, phenylamide-resistant isolates belonging to the old population have been reported (Goodwin *et al.*, 1996; McLeod *et al.*, 2001). The proportion of metalaxyl-resistant isolates fluctuates from year to year and within season (Gisi and Cohen, 1996). In the 1990s, resistance levels remained more or less stable in all European countries (Gisi and Cohen, 1996).

A previous study of genotypic and phenotypic diversity of *P. infestans* collected from Estonia in 2002 and 2003 (Runno-Paurson *et al.*, 2009) showed that all isolates in that collection belonged to the 'new population' (as identified via allozyme genotypes, mtDNA haplotypes and mating type). The results indicated that Estonian population of *P. infestans* is diverse, with potential for sexual recombination. Whereas in the previous study isolates were collected only from three regions (central, eastern and southern), more regions were included in the present study in order to provide more detail on the Estonian population of *P. infestans*.

The main objective of this study was to characterize the population of *P. infestans* in Estonia for metalaxyl sensitivity, virulence, mating type, RFLP fingerprint and mtDNA haplotype, to find answers to the following questions: (i) does the mating type ratio in Estonia suggest occur-

rence of sexual reproduction?; (ii) is phenotypic and genotypic variation at the same level as in other European countries as indicated by virulence and DNA-fingerprints?; (iii) are there any indications of oospore-derived epidemics? In addition, the impacts of time and regions were studied.

Materials and methods

Collection of isolates. Potato leaves infected by *P. infestans* were collected from Estonia during the period 2004-2007 from 25 sites (small-scale conventional, large-scale conventional productions and untreated experimental field plots) in six regions of Estonia (Table 1). Conventional producers were divided into two groups. On the small scale, farmers used seed potatoes of uncertain quality and did not follow the rotation rules. Chemical late blight treatments were applied occasionally, varying from no sprays to 1-4 sprays. On the large scale, farmers used high-quality certified seed potatoes, planted potatoes no more frequently than every 3rd year (with some exceptions) and applied fungicide 6-7 times per season, while sometimes the fungicide was applied as often as 11 times/season. In experimental plots at Jõgeva Plant Breeding Institute (four sites) diverse cultivars and breeding lines were used, and the quality of seed potatoes was also diverse.

Isolates were collected at the beginning of outbreaks in all years. Ten to thirty blighted leaves, each with a single lesion (one per plant), were collected (Table 1). The plants were selected by randomising the distance from field edges, and from each plant the blighted leaf was also randomly chosen, excluding those that had several or no lesions.

Isolations were carried out as described in Runno-Paurson *et al.* (2009). All phenotypic tests were done in October-November of the year of isolation. Mitochondrial DNA haplotype analyses were done in November-December 2005.

All 432 isolates were tested for virulence; 424 were tested for mating type; 412 were tested for response to metalaxyl. Subgroups of 57 isolates were tested for mtDNA haplotype and for RG57 fingerprints (Table 1).

Phenotypic analyses. The resistance to metalaxyl of all 412 isolates was tested using a modification of the floating leaflet method (Hermansen

et al., 2000) as described in Runno-Paurson *et al.* (2009). The specific virulence of each isolate was determined by using Black's differential set of potato genotypes containing resistance genes R1-R11 (Malcolmson and Black, 1966) (provided by the Scottish Agricultural Science Agency). Laboratory procedures were as described in Runno-Paurson *et al.* (2009).

Mating types were determined according to Runno-Paurson *et al.* (2009). Tester isolates were the same as those described in Lehtinen *et al.* (2006).

Neutral marker assessment. A subset of 57 isolates was RFLP fingerprinted using Goodwin *et al.* (1992a) technique, as described by Runno-Paurson *et al.* (2009). The DNA fingerprints of the Estonian isolates were determined by comparing their patterns with those of three reference isolates (belonging to the US-1, US-8 and US-17 clonal lineages).

The mitochondrial DNA (mtDNA) haplotype of each of the 57 isolates in the subset was determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), a variation of the method by Griffith and Shaw (1998) as described by Runno-Paurson *et al.* (2009).

Data analysis. The isolates were described as a multilocus genotype consisting of DNA fingerprint bands, mtDNA haplotype and mating type. Genotypic diversity as well as race diversity was calculated with the normalized Shannon diversity index (Sheldon, 1969): $H_s = - \sum g_i \ln g_i / \ln N$, where g_i is the frequency of the i th multilocus genotype and N the sample size. The normalized 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 the SAS/STAT version 9.1 (SAS Institute, Cary, USA). Differences in the prevalence of the two mating types of *P. infestans* isolates between study sites and years 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 years, and also between different haplotypes. Logistic analyses were also used to test for associations between each of the polymorphic RG57

bands and metalaxyl sensitivity, mating type and mtDNA haplotype. The dependence of specific virulence (percent of isolates that show virulence against particular R-genes) on years, sites, field types and R-genes was analyzed with type III ANOVA. "Site" was treated as a random variable and nested within "field type". Tukey HSD post-hoc tests ($\alpha = 0.05$) were applied to find specific differences between sites and R-genes. In all analyses, "year" was treated as a categorical variable. See Runno-Paurson *et al.* (2009) for more details.

Results

Resistance to metalaxyl. In total, 412 isolates were screened for resistance to metalaxyl. Over the four years, 37% of isolates were resistant to metalaxyl, 25% were intermediate and 37% were classified as sensitive. Of the metalaxyl-resistant strains, 37% were A1 mating type, 40% were A2 mating type and 23% were self-fertile.

The proportion of metalaxyl-resistant isolates differed between sites ($\chi^2 = 42.20$, $df = 11$, $p < 0.001$) and years ($\chi^2 = 61.57$, $df = 3$, $p < 0.001$). The proportion of metalaxyl-resistant isolates ranged from 0 to 81% depending on site. In 2004, 56% of all isolates were resistant to metalaxyl, whereas in 2007, most isolates (Table 2) were sensitive to metalaxyl. Metalaxyl-resistant strains were absent from only three fields (Enge 2007, Naha 2005 and Vönnu 2006 - where metalaxyl fungicide was not used). Significant differences between potato cropping systems were not observed ($\chi^2 = 0.05$, $df = 1$, $p = 0.82$). There was a strong association between metalaxyl resistance and sites where metalaxyl-containing fungicides had been applied ($\chi^2 = 9.24$, $df = 1$, $p = 0.0024$). Over four years, from crops known to have been sprayed with fungicide containing a metalaxyl, 48% of isolates were resistant, while isolates collected from potatoes which had not been metalaxyl-treated, only 33% of isolates were resistant (Table 3).

Virulence. All known virulence factors (to overcome genes R1-R11) were found in this collection. We found significant differences in the frequencies of specific virulences among sites and years ($F_8 = 9.41$, $p < 0.001$) and virulences to specific R-genes also differed ($F_{10} = 234.31$, $p < 0.001$). No main effect of cropping systems was detected. Almost all isolates were virulent on differentials with genes R1, R3, R4, R7, R10 and R11.

Least frequent was virulence against R9 (3%). Virulence against R5 was also infrequent (17%) (Fig. 1). Most of the isolates (>96%) were able to overcome four or more R-genes. Eighty-seven different pathotypes were detected (Table 4). The three most common pathotypes made up 39% (Table 4) of isolates in the sample. More than half of the races (>65%) were detected only once. The virulence complexity (average number of R-genes overcome) ranged from 4.8 for isolates from Laheotsa 2004 to 8.9 in isolates from Jõgeva 2005 (Table 5). The most common pathotypes were 1.3.4.7.10.11 and 1.2.3.4.7.10.11 (Table 4). The normalized Shannon diversity index was 0.54.

Mating type. Of the 424 isolates tested, 64% were of A1 mating type, 33% were A2 mating type and 3% were self-fertile. Both mating types were found at each sample site (25 sites). The frequency of A2 ranged from 3% to 71%. In 2004 and 2007, almost a half (44% and 48% respectively) of the isolates tested were A2 mating type. The proportion of the A2 mating type decreased from 44% in 2004 to 13% in 2006 and increased again to 48% in 2007. There were considerable differences between sites ($\chi^2 = 28.68$, $df = 10$, $p = 0.0014$) and years ($\chi^2 = 26.28$, $df = 3$, $p < 0.001$) in the occurrence of the two mating types. The 263 A1 isolates tested included 97 that were resistant to metalaxyl, 72 that were intermediate, and 94 that were sensitive. Among the 134 A2 mating type isolates, 53 were resistant, 28 were intermediate, and 53 were sensitive. The proportion of A2 mating type was higher among isolates from small-scale conventional fields (39%) than from large-scale conventional fields (26%) ($\chi^2 = 7.05$, $df = 1$, $p = 0.0079$).

RG57 analysis. Based on a worldwide collection of isolates, the RG57 probe can detect (presence/absence) a total of 25 restriction fragments/bands (Goodwin *et al.*, 1992a). In the subset of 57 isolates, nine of these bands were not detected (bands 4, 8, 11, 12, 15, 17, 19, 22 and 23), while five were present in all isolates (bands 13, 14, 20, 24 and 25). The remaining eleven bands (bands 1, 2, 3, 5, 6, 7, 9, 10, 16, 18, 21) were polymorphic. A total of 21 different fingerprints were detected. The four most common fingerprints I, XX, XXI and XXII (Table 6) represented more than half the isolates (67%). Fourteen unique fingerprints were detected at nine sites, but mostly among isolates collected in 2005.

Mitochondrial DNA haplotype. Of the four known mitochondrial DNA haplotypes, three were found. Among 57 isolates tested, 30 were

Ia haplotype (51%), 24 were IIa haplotype (42%) and 3 were IIb haplotype (7%). For the 37 A1 isolates 20 were Ia, 14 were IIa and 3 were IIb. For the 20 A2 isolates 10 were Ia and 10 were IIa. There were differences in frequency of Ia and IIa between sites. Only IIa haplotype isolates were detected in Enge 2004, Kalsa 2005 and Kambja 2004.

Multilocus genotypes. Thirty-three multilocus genotypes were identified, of which 27 (81%) were detected only once, most of these collected in 2005 (Table 6). All belonged to the 'new' population of the pathogen that displaced the US-1 clonal lineage (A1 mating type, Ib mtDNA haplotype) (Spielman *et al.*, 1991; Lebreton and Andrivon, 1998). Genotypic diversity measured by the normalized Shannon index was 0.79. Three genotypes were common to Estonian samples: the most frequent, EE-1 was found 8 times, EE-2 was found 6 times.

Associations between markers. Most of the RG57 fingerprints were not associated with a single mtDNA haplotype (Table 9). There was no association between metalaxyl resistance and mtDNA haplotype ($\chi^2 = 1.66$, $df = 3$, $p = 0.65$). Of the 25 polymorphic RG57 bands, one polymorphic band (16) was significantly associated with metalaxyl tolerance ($\chi^2 = 8.28$, $df = 2$, $p = 0.016$). No associations were found between RG57 fingerprints, metalaxyl resistance or mating types.

Discussion

Comparison of data from the current study with previous data (Runno-Paurson *et al.*, 2009) showed that the proportion of metalaxyl-resistant isolates of *P. infestans* remains in the same range as it was in Estonia in 2002-2003. Similar values have been found in recent years in other European countries (Hermansen *et al.*, 2000; Bakonyi *et al.*, 2002a, 2002b; Day *et al.*, 2004; Nagy *et al.*, 2006; Lehtinen, *et al.*, 2007; Avendaño Córcoles, 2007).

The proportion of isolates with intermediate resistance to metalaxyl was high in the Estonian population during the period of 2002-2006; similar situation was noted in the UK by Cooke *et al.* (2003) and Day *et al.* (2004). However, by the year 2007 the proportion of isolates with intermediate resistance in the Estonian population had decreased to 6%.

The proportion of metalaxyl-resistant isolates in the Estonian population has fluctuated considerably throughout the recent years. From a level of about 20-35% in 2002 and 2003 (Runno and Koppel 2006) it increased to 56% in 2004. This peak was followed by a decrease and among the isolates collected in 2006 and 2007, metalaxyl-resistant isolates were, again, in the minority (Table 2). Part of the reason for such a decrease (which was more pronounced in 2007) could be that in 2007 the weather conditions were not favourable for late blight development and the pathogen occurred quite late in the season, so farmers/growers did not treat crop so frequently and did not use metalaxyl fungicides as often as in other years. Although the proportion of metalaxyl-resistant isolates fluctuates from year to year it is still clear that this proportion has remained substantially high throughout the whole monitoring period. The most likely reason for the fluctuations is the changing intensity of the use of metalaxyl-containing fungicides. The situation is very similar in the Finnish population (Lehtinen *et al.*, 2007), where the extent of metalaxyl-resistant isolates has increased and the use of phenylamide fungicides is very common.

In the current study as might be expected, resistance was more often found in potato fields known to have been sprayed with phenylamide containing fungicides, 48% of isolates were resistant, whereas from fields which had not been phenylamide-treated, only 33% of isolates were resistant (Table 3). Metalaxyl was used mostly on large scale conventional fields.

Trials of late blight control with metalaxyl containing fungicides carried out in 2003-2005 (Runno and Koppel 2006) at Jõgeva Plant Breeding Institute indicated that metalaxyl provided high protection under moderate pathogen pressure, but showed lowered efficacy only in conditions of extreme late blight pressure in 2004 when the foliage was already infected during the period when metalaxyl was used.

Results showed that in spite of the occurrence of resistant strains, the use of metalaxyl-containing fungicides is still effective. By following the application instructions for metalaxyl fungicides it is possible to restrict the selection for metalaxyl-resistant strains. Therefore metalaxyl could be used effectively for control of potato late blight a maximum of two times at the start of the fungicide treatments.

Comparing the current results with previous results in 2002-2003 (Runno-Paurson *et al.*, 2009), the frequency of virulence against R5 had increased from 5% to 17% and frequency against R9 had decreased from 14% to 3%.

The race 1.3.4.7.10.11 was the most common among Estonian isolates as it is in Finland, Norway, Denmark and Sweden (Hermansen *et al.*, 2000, Lehtinen *et al.*, 2007, Lehtinen *et al.*, 2008), in France and Switzerland (Knapova and Gisi, 2002), in Poland (Zimnoch-Guzowska, 1999) and in Austria (Avendaño Córcoles, 2007). Complex races are common in Estonian populations, a similar situation to those in Poland (Śliwka *et al.*, 2006) and Russia (Elansky *et al.*, 2001). The mean number of virulence genes per isolate increased from 6.3 in 2002-2003 to 6.9 in 2004-2007. A similar increase has also occurred in Finland and Norway (Lehtinen *et al.*, 2008).

In the current study the four most common pathotypes formed almost half (46%) of all isolates (Table 4), but in contrast to previous studies most of the pathotypes appeared only once, and the three most common pathotypes comprised only 16.8% of the population. Pathotype diversity calculated by the normalised Shannon diversity index also showed lower values (0.54) than in 2002-2003 when it was very high (0.92). The index value showed the same range as other European populations in Austria (0.56) (Avendaño Córcoles, 2007), Finland (0.35) and Norway (0.44) (Hermansen *et al.*, 2000).

The average percentage of A2 mating type in the current study was 33%, which is similar to results in previous studies in 2002-2003 (Runno-Paurson *et al.*, 2009). There have been reports from several European countries where the A1:A2 ratio is lower (Brurberg *et al.*, 1999; Hermansen *et al.*, 2000; Bakonyi *et al.*, 2002a, 2002b; Cooke *et al.*, 2006; Lehtinen, *et al.*, 2007). A higher proportion of the A2 mating type has been found in Austria, The Netherlands and Poland (Avendaño Córcoles, 2007; Zwankhuizen *et al.*, 2000; Śliwka *et al.*, 2006).

Both mating types were detected in all fields studied. It may indicate that blight epidemics are severe both on large conventional farms and in untreated experimental fields. This situation with continuous potato cropping in small-scale conventional fields increases the risk of oospore-derived infections and may cause more early attacks and consequent yield

loss. The presence of both mating types in the same field indicates that oospores would be produced in potato foliage (Turkensteen *et al.*, 2000) and may change the epidemiology substantially. Sexual reproduction increases genetic diversity and leads to soil contamination with oospores, and associated early infection. Indeed, in 2004, symptoms indicated that the infection was probably caught from oospore-contaminated soil. Therefore more emphasis needs to be put on crop rotation (Turkensteen *et al.*, 2000).

In this study we noted differences between potato production systems. The proportion of A2 mating type was higher among isolates collected from experimental fields (40%) and small-scale conventional farm fields (39%) than from large-scale conventional fields (26%). Differences on this scale could signify higher genetic diversity among populations collected from experimental and small-scale productions, as there is a higher risk of sexual reproduction. However, our data may result from a sampling bias.

This study uncovered several new RG57 genotypes of *P. infestans* not previously reported. Among numerous RG57 fingerprints only three (I, XX and XXI) occurred in 2004 and again in 2005. Most of the 21 RG57 fingerprints are apparently unique to Estonia. Comparing the results of this study with other European studies, shows similarities in fingerprints from Russia, Finland, Norway, the Netherlands, Great Britain, and Belgium. The most common fingerprint in Estonia I, was identical with the Russian fingerprint MO-12 (Moscow region) (Elansky *et al.*, 2001), the Norwegian fingerprint N-27 (Brurberg *et al.*, 1999) and the British fingerprint RF060 (Day *et al.*, 2004) (Table 6). The fingerprint XX was identical with the Dutch fingerprint NL-86 (Zwankhuizen *et al.*, 2000), the fingerprint XXI with the Russian fingerprint MO-5 (Elansky *et al.*, 2001); XXIII was identical with the Russian fingerprint MO-17 (Elansky *et al.*, 2001) and the British fingerprint RF015 (Day *et al.*, 2004); XXXV was identical with the British fingerprint RF006 and the most common Northern Ireland fingerprint NI-1 (Day *et al.*, 2004, Cooke *et al.*, 2006).

The three mtDNA haplotypes (Ia, IIa and IIb) were found among our isolates. The Ib haplotype, associated with the old clonal *P. infestans* populations, was not found. Haplotype IIb was not found previously in Estonia. Interestingly, among isolates collected from metalaxyl-treated

crops the extent of IIb haplotype, which is rare in Europe, was 23%. In the current and previous studies, both haplotypes were found in almost equal proportions. A high proportion of Ia haplotype (74%) was found in northern Estonia (Runno-Paurson, unpublished data). A high proportion of Ia haplotype has also been observed in Poland, England, Scotland, Wales, The Netherlands and France (Lebreton and Andrivon *et al.*, 1998; Cooke *et al.*, 2003; Lebecka *et al.*, 2007).

Our study indicates that the Estonian population of *P. infestans* is diverse, having a large number of multilocus genotypes. The high and stable frequency of A2 isolates and the occurrence of both mating types in the same field indicates a potential for sexual recombination and spread of fungicide resistance. More information is needed to clarify the role of oospores in the epidemiology of *P. infestans* in Estonia.

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References

- Andrivon D., 1994. Races of *Phytophthora infestans* in France, 1991-1993. *Potato Research* **37**: 279-286.
- Avendaño Córcoles J., 2007. Survey of *Phytophthora infestans* population in Austria based on phenotypic and molecular markers. Ph.D. Thesis, University of Natural Resources and Applied Life Sciences, Vienna, Austria.
- Bakonyi J., Ládai M., Dula T., Érsek T., 2002a. Characterisation of isolates of *Phytophthora infestans* from Hungary. *European Journal of Plant Pathology* **108**: 139-146.

- Bakonyi J., Heremans B., Jamart G., 2002b. Characterization of *Phytophthora infestans* isolates collected from potato in Flanders, Belgium. *Phytopathology* **150**: 512-516.
- Brurberg M.B., Hannukala A., Hermansen A., 1999. Genetic variability of *Phytophthora infestans* in Norway and Finland as revealed by mating type and fingerprint probe RG57. *Mycological Research* **12**: 1609-1015.
- Carlisle D.J., Cook L.R., Watson S., Brown A.E., 2002. Foliar aggressiveness of Northern Ireland isolates of *Phytophthora infestans* on detached leaflets of three potato cultivars. *Plant Pathology* **51**: 424-434.
- Cooke D.E.L., Young V., Birch P.R.J., Toth R., Gourlay F., Day J.P., Carnegie S. F., Duncan J. M., 2003. Phenotypic and genotypic diversity of *Phytophthora infestans* populations in Scotland (1995-97). *Plant Pathology* **52**: 181-192.
- Cooke L.R., Carlisle D.J., Donaghy C., Quinn M., Perez F.M., Deahl K.L., 2006. The Northern Ireland *Phytophthora infestans* population 1998-2002 characterized by genotypic and phenotypic markers. *Plant Pathology* **55**: 320-330.
- Davidse L.C., Looijen D., Turkensteen L.J., van der Val D., 1981. Occurrence of metalaxyl-resistance strains of *Phytophthora infestans* in Dutch potato fields. *Netherlands Journal of Plant Pathology* **87**: 65-68.
- Day J.P., Wattier R.A.M., Shaw D.S., Shattock R.C., 2004. Phenotypic and genotypic diversity in *Phytophthora infestans* on potato in Great Britain, 1995-98. *Plant Pathology* **53**: 303-315.
- Drenth A., Tas I.C.Q., Govers F., 1994. DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. *European Journal of Plant Pathology* **100**: 97-107.
- Drenth A., Goodwin S.B., Fry W.E., Davidse L.C., 1993. Genotypic diversity of *Phytophthora infestans* in The Netherlands revealed by DNA polymorphisms. *Phytopathology* **83**: 1087-1092.
- Dowley L.J., O'Sullivan E., 1981. Metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. *Potato Research* **24**: 417-421.
- Elansky S., Smirnov A., Dyakov Y., Dolgova A., Filippov A., Kozlovski B., Kozlovskaja I., Russo P., Smart C., Fry W., 2001. Genotypic analysis of Russian isolates of *Phytophthora infestans* from the Moscow region, Siberia and Far East. *Phytopathology* **149**: 605-611.

- Fry W.E., Goodwin S.B., 1997. Re-emergence of potato and tomato late blight in the United States. *Plant Disease* **81**: 1349-1357.
- Fry W.E., Goodwin S.B., Dyer A.T., Matuszak J.M., Drenth A., Tooley P.W., Sujkowski L.S., Koh Y.J., Cohen B.A., Spielman L.J., Deahl K.L., Inglis D.A., Sandlan K.P., 1993. Historical and recent migrations of *Phytophthora infestans*: chronology, pathways, and implications. *Plant Disease* **77**: 653-661.
- Fry W.E., Goodwin S.B., Matuszak J.M., Spielman L.J., Milgroom M.G., Drenth A., 1992. Population genetics and intercontinental migrations of *Phytophthora infestans*. *Annual Review of Phytopathology* **30**: 107-29.
- Gisi U., Cohen Y., 1996. Resistance to phenylamide fungicides: A case study with *Phytophthora infestans* involving mating type and race structure. *Annual Review of Phytopathology* **34**: 549-572.
- Goodwin S.B., Sujkowski L.S., Fry W.E., 1996. Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and western Canada. *Phytopathology* **85**: 793-800.
- Goodwin S.B., Drenth A., Fry W.E., 1992a. Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Current Genetics* **22**: 107-115.
- Goodwin S.B., Spielman L.J., Matuszak J.M., Bergeron S.N., Fry W.E., 1992b. Clonal diversity and genetic differentiation of *Phytophthora infestans* in northern and central Mexico. *Phytopathology* **82**: 955-961.
- Griffith G.W., Shaw D.S., 1998. Polymorphisms in *Phytophthora infestans*: Four mitochondrial haplotypes are detected after PSR amplification of DNA from pure cultures or from host tissue. *Applied and Environmental Microbiology* **64**: 4007-4014.
- Hermansen A., Hannukkala A., Hafskjold Naerstad R., Brurberg M., 2000. Variation in populations of *Phytophthora infestans* in Finland and Norway: mating type, metalaxyl resistance and virulence phenotype. *Plant Pathology* **49**: 11-22.
- Knapova G., Gisi U., 2002. Phenotypic and genotypic structure of *Phytophthora infestans* populations on potato and tomato in France and Switzerland. *Plant Pathology* **51**: 641-653.

- Lebecka R., Śliwka J., Sobkowiak S., Zimnoch-Guzowska, E., 2007. *Phytophthora infestans* population in Poland. *PPO-Special Report no. 12*: 155-159.
- Lebreton L., Andrivon D., 1998. French isolates of *Phytophthora infestans* from potato and tomato differ in phenotype and genotype. *European Journal of Plant Pathology* **104**: 583-94.
- Lehtinen A., Hannukkala A., Andersson B., Hermansen A., Le V.H., Naerstad R., Brurberg M.B., Nielsen B.J., Hansen J.G., Yuen J., 2008. Phenotypic variation in Nordic populations of *Phytophthora infestans* in 2003. *Plant Pathology* **57**: 227-234.
- Lehtinen A., Hannukkala A., Rantanen T., Jauhiainen L., 2006. Phenotypic and genetic variation in Finnish potato-late blight populations, 1997-2000. *Plant Pathology* **56**: 480-491.
- Malcolmson J.F., Black W., 1966. New R genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica* **15**: 199-203.
- McLeod A., Denman S., Sadie A., Denner F.D.N., 2001. Characterisation of South African isolates of *Phytophthora infestans*. *Plant Disease* **85**: 287-291.
- Nagy Z.Á., Bakonyi J., Virag Som, Érsek T., 2006. Genetic Diversity of the population of *Phytophthora infestans* in Hungary. *Acta Phytopathologica et Entomologica Hungarica* **41**: 53-67.
- Runno E., Koppel M., 2006. The question of metalaxyl resistance on late blight fungus in Estonia. *Agronomy Research* **4**: 341-344.
- Runno-Paurson E., Fry W.E., Myers K.L., Koppel M., Mänd M., 2009. Characterization of *Phytophthora infestans* isolates collected from potato in Estonia during 2002-2003. *European Journal of Plant Pathology* **124**: 565-575.
- Shattock R.C., 2002. *Phytophthora infestans*: populations, pathogenicity and phenylamides. *Pest Management Science* **58**: 944-50.
- Shattock R.C., Tooley P.W., Fry W.E., 1986. Genetics of *Phytophthora infestans*: Determination of recombination, segregation, and selfing by isozyme analysis. *Phytopathology* **76**: 410-413.
- Sheldon A.L., 1969. Equitability indices: Dependence on the species count. *Ecology* **50**: 466-467.

- Śliwka J., Sobkowiak S., Lebecka R., Avendano Córcoles J., Zimnoch-Guzowska E., 2006. Mating type, virulence, aggressiveness and meta-laxyl resistance of isolates of *Phytophthora infestans* in Poland. *Potato Research* **49**: 155-166.
- Spielman L.J., Drenth A., Davidse L.C., Sujkowski L.J., Gu W., Tooley P.W., Fry W.E., 1991. A second world-wide migration and population displacement of *Phytophthora infestans*? *Plant Pathology* **40**: 422-430.
- Tooley P.W., Fry W.E., Villareal M.J., 1985. Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. *Journal of Heredity* **76**: 431-435.
- Turkensteen L.J., Flier W.G., Wanningen R., Mulder A., 2000. Production, survival and infectivity of oospores of *Phytophthora infestans*. *Plant Pathology* **49**: 688-696.
- Vorobyeva Yu.V., Gridnev V.V., Bashaeva E.G., Pospelova L.A., Kvasnyuk N.Ya., Kuznetsova L.N., Shemyakina V.P., Morozova E.V., Zherbtsova L.N., Rozalyeva V.V., 1991. On the occurrence of the A2 mating type isolates of *Phytophthora infestans* (Mont.) de Bary in the USSR. *Mikologija i fitopatologija* pp.62-67.
- Zimnoch-Guzowska E., 1999. Late blight and blight research in Central and Eastern Europe. In: Crissman, L., C. Lizárraga (eds). *Proceedings of the Global Initiative on Late Blight Conference: A Threat to Global Food Security, Quito 1999* **1**: 9-14,
- Zwankhuizen M.J., Govers F., Zadoks J.C., 2000. Inoculum sources and genotypic diversity of *Phytophthora infestans* in Southern Flevoland, the Netherlands. *European Journal of Plant Pathology* **106**: 667-680.

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Table 1. Origin and characteristics of *Phytophthora infestans* isolates collected from Estonia (2004-2007)

Region	Year	Number of isolates tested for							
		Number of sites	Number of isolates	Mating type	Metalaxyl resistance	Virulences	mtDNA haplotype	RG57 fingerprints	
Northern	2004	1	17	17	17	17	4	4	
	2005	1	10	10	10	10	5	4	
Central	2004	1	22	22	20	22	4	4	
	2005	1	25	23	23	25	5	5	
	2007	1	19	15	15	19	0	0	
Eastern	2004	1	19	18	18	19	4	4	
	2005	1	18	17	17	18	5	5	
	2006	2	45	45	44	45	0	0	
South-eastern	2007	1	23	21	21	23	0	0	
	2004	1	24	24	20	24	4	6	
	2005	3	50	50	49	50	13	15	
	2006	3	46	46	44	46	0	0	
	2007	2	31	28	28	31	0	0	
South-western	2004	1	13	14	13	13	4	4	
	2006	1	13	13	13	13	0	0	
	2007	1	16	16	16	16	0	0	
Southern	2004	3	41	45	44	41	11	8	
Total		25	432	424	412	432	59	59	

Legend to the figure

Table 2. Metalaxyl resistance^a among isolates of *Phytophthora infestans* in Estonia (2004-2007)

Year	Percentage of isolates			Isolates tested (n)
	S (%)	I (%)	R (%)	
2004	23.5	20.5	56.1	132
2005	15.2	41.4	43.4	99
2006	45.5	30.7	23.8	101
2007	78.8	6.3	15.0	80
Total	37.6	25.2	37.1	412

^aS, metalaxyl-sensitive; I, intermediate metalaxyl-sensitive; R, metalaxyl-resistant.

Table 3. Metalaxyl-resistance^a among isolates from fields with or without phenylamide fungicide treatments (years 2004-2007) and results of logistic analysis

Field management	Number (and percentage) of isolates		
	S	I	R
Treated with metalaxyl n = 106	30 (28.3)	25 (23.6)	51 (48.1)
Not metalaxyl-treated n = 306	125 (40.8)	79 (25.8)	102 (33.3)
Results of logistic analysis			
	DF	Chi-square	P
	1	9.24	p = 0.0024

^aS, metalaxyl-sensitive; I, intermediate-metalaxyl sensitive; R, metalaxyl-resistant.

Table 4. Number of isolates of different races among isolates of *Phytophthora infestans* from Estonia (2004-2007)

Race	Number of Virulence factors	Number of isolates	Race	Number of Virulence factors	Number of isolates
1.2.3.4.5.6.7.8.9.10.11	11	6	3.6.7.10.11	5	1
1.2.3.4.5.6.7.9.10.11	10	1	3.7.8.10.11	5	1
1.2.3.4.5.7.8.9.10.11	10	1	1.3.4.10.11	5	2
1.2.3.4.6.7.8.9.10.11	10	4	1.3.4.7.11	5	2
1.2.3.4.5.6.7.8.10.11	10	5	3.4.7.10.11	5	2
1.3.4.5.6.7.8.10.11	9	3	1.4.7.10.11	5	3
1.2.3.4.5.6.7.10.11	9	10	1.3.7.10.11	5	5
1.2.3.4.5.7.8.10.11	9	17	1.3.4.11	4	1

Table 4. (Continued)

Race	Number of Virulence factors	Number of isolates	Race	Number of Virulence factors	Number of isolates
1.2.3.4.6.7.8.10.11	9	26	1.3.7.10	4	1
1.2.3.4.6.8.10.11	8	1	1.3.7.11	4	1
1.3.4.5.6.8.10.11	8	1	1.4.7.10	4	1
1.3.4.5.6.7.10.11	8	3	1.4.7.11	4	1
1.3.4.5.7.8.10.11	8	4	1.7.10.11	4	1
1.3.4.6.7.8.10.11	8	4	1.7.8.11	4	1
1.2.3.4.5.7.10.11	8	11	2.3.4.7	4	1
1.2.3.4.6.7.10.11	8	30	2.4.7.10	4	1
1.2.3.4.7.8.10.11	7	39	2.7.10.11	4	1
1.2.3.5.7.8.11	7	1	3.4.5.7	4	1
1.2.3.7.8.10.11	7	1	3.6.7.11	4	1
1.2.4.6.7.10.11	7	1	4.6.7.11	4	1
1.3.4.7.9.10.11	7	1	6.7.10.11	4	1
1.3.6.7.8.10.11	7	1	3.4.7.10	4	2
2.3.4.7.8.10.11	7	1	1.3.7	3	1
3.4.5.7.10.11	7	1	3.6.7	3	1
1.2.3.6.7.10.11	7	3	4.6.7	3	1
2.3.4.7.10.11	7	3	4.7.10	3	1
1.3.4.5.7.10.11	7	8	1.3.11	3	1
1.3.4.6.7.10.11	7	10	1.7.11	3	1
1.3.4.7.8.10.11	7	34	4.7.11	3	1
1.2.3.4.7.10.11	6	46	6.7.11	3	1
1.2.3.4.7.11	6	1	3.8.11	3	1
1.2.3.6.10.11	6	1	3.10.11	3	1
1.3.4.7.8.11	6	1	1.10.11	3	2
1.3.7.8.10.11	6	1	7.10.11	3	3
1.4.6.7.10.11	6	1	4.7	2	2
1.4.7.8.10.11	6	1	6.10	2	1
2.3.6.7.10.11	6	1	1.11	2	1
1.2.4.7.10.11	6	2	3.11	2	1
1.2.3.7.10.11	6	3	10.11	2	1
1.3.4.7.10.11	5	81	3.10	2	2
1.3.4.7.10	5	1	11	1	1
2.3.4.7.11	5	1	10	1	1
2.4.7.10.11	5	1	7	1	2
Total number of isolates					432
Total number of races					87

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	Virulence to resistance gene														Mean number of	No of tested
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11					
Antsla 2004	87	40	87	80	20	47	80	53	0	87	100	6.8	15			
Enge 2004	100	38	100	100	15	15	100	38	0	100	100	7.1	13			
Enge 2006	100	23	92	100	8	8	100	38	0	92	100	6.6	13			
Enge 2007	81	69	94	81	13	19	94	50	6	75	88	6.7	16			
Inglise 2004	86	5	91	77	9	27	91	9	0	86	91	5.7	22			
Inglise 2006	92	48	96	100	36	16	96	48	8	96	96	7.3	25			
Inglise 2007	100	26	89	84	11	16	100	11	0	95	95	6.3	19			
Jõgeva 2004	84	63	89	84	5	32	100	42	11	95	100	7.1	19			
Jõgeva 2005	100	94	100	100	56	67	100	56	17	100	100	8.9	18			
Jõgeva 2006	90	60	83	93	7	53	97	47	3	83	100	7.2	30			
Jõgeva 2007	100	78	91	96	17	35	100	43	0	100	96	7.6	23			
Jõusa 2006	100	27	100	100	20	7	100	20	7	100	100	6.8	15			
Kalsa 2005	95	74	95	100	5	37	95	32	0	95	95	7.2	19			
Kalsa 2006	100	53	93	100	7	20	100	47	0	100	100	7.2	15			
Kambja 2004	100	60	100	73	13	0	100	20	0	100	100	6.7	15			
Laheotsa 2004	53	29	82	59	6	24	82	0	0	71	71	4.8	17			
Laheotsa 2005	100	90	100	100	70	20	100	30	20	100	100	8.3	10			
Naha 2005	90	70	90	90	10	60	100	60	0	100	100	7.7	10			
Naha 2006	81	13	81	88	6	6	94	31	0	94	88	5.8	16			
Naha 2007	100	31	100	100	19	6	94	19	0	100	100	6.7	16			
Paalimäe 2004	82	82	91	73	0	64	91	9	0	100	100	6.9	11			

Table 5. (Continued)

Virulence to resistance gene	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	Mean number of	No of tested
Võnnu 2004	63	29	79	71	4	38	92	33	0	79	83	5.7	24
Võnnu 2005	100	86	95	95	57	29	95	57	5	100	95	8.1	21
Võnnu 2006	93	47	80	87	7	27	93	60	0	100	100	6.9	15
Võnnu 2007	100	27	100	100	13	7	100	67	0	100	100	7.1	15
Total												6.9	432

Table 6. Genotypes, phenotypes and locations of Estonian isolates of *Phytophthora infestans*

Multilocus genotype	RG57 fingerprinta	RG57 genotypb	Mating type	mtDNA haplo-type	Metal-axyl re-sistancec	Number of iso-lates	Number of sites	Year	Regiond	Field type
EE-1	1010101001001101000110011	I	A1	Ia	R, S	8	4	2004-05	C, N, S	S, L
EE-2	1010101001001101000110011	I	A1	IIa	S, R, I	6	4	2004-05	E, SE, SW	E, L, S
EE-27	1010101001001101000110011	I	A1	IIb	S	1	1	2005	C	L
EE-3	1010101001001101000110011	I	A2	Ia	S, R	2	2	2004-05	C, S	L, S
EE-28	1010101001001101000110011	I	A2	IIa	S, R	4	2	2004-05	E, S	E, S
EE-29	1010101001001100000110011	XX	A1	Ia	I, R, S	4	3	2004-05	N, SE	L, S
EE-30	1010101001001100000110011	XX	A1	IIa	R	2	1	2004	SE	S
EE-31	1010101001001100000110011	XX	A2	Ia	S	1	1	2004	E	E
EE-32	1010101001001100000110011	XX	A2	IIa	R	1	1	2004	C	L
EE-33	1000101001001101000110011	XXI	A1	IIa	I	1	1	2005	SE	L
EE-34	1000101001001101000110011	XXI	A1	IIb	R	1	1	2004	C	L
EE-35	1000101001001101000110011	XXI	A2	IIa	S	2	2	2004-05	SE, SW	L
EE-36	1010001001001101000110011	XXII	A1	IIa	I	1	1	2005	C	L
EE-37	1010001001001101000110011	XXII	A2	Ia	I	4	1	2004	N	L
EE-38	10101011001101000110011	XXIII	A1	IIa	I	1	1	2004	C	L
EE-39	10101011001101000110011	XXIII	A2	Ia	I	1	1	2004	E	E
EE-40	10101011001101000110011	XXIII	A2	IIa	R	1	1	2004	E	E
EE-41	1110101001001100000110011	XXIV	A1	Ia	R	1	1	2005	SE	S
EE-43	1110101001001101000110011	XXV	A1	Ia	R	1	1	2005	SE	S
EE-44	1110101001001101000110011	XXV	A1	IIb	S	1	1	2005	SE	S

Table 6. (Continued)

Multilocus genotype	RG57 fingerprinta	RG57 genotypeb	Mating type	mtDNA haplo-type	Metal-axyl re-sistrancec	Number of iso-lates	Number of sites	Year	Regiond	Field type
EE-45	1110001001001101000110011	XXVI	A1	Ia	I	1	1	2005	N	L
EE-46	1100110001001101000110011	XXVII	A1	Ia	I	1	1	2005	SE	L
EE-47	101011011001101000110011	XXVIII	A1	Ia	R	1	1	2004	E	E
EE-42	101011001001101000110011	XXIX	A1	Ia	I	1	1	2005	E	E
EE-48	1010101001001101000100011	XXX	A1	IIa	R	1	1	2005	SE	L
EE-49	1010100001001101000110011	XXXI	A1	Ia	I	1	1	2005	SE	S
EE-50	1000101001001100000110011	XXXII	A1	IIa	I	1	1	2004	C	L
EE-51	1000101000001101000110011	XXXIII	A2	IIa	I	1	1	2005	SE	L
EE-53	1000100011001100000110011	XXXIV	A1	Ia	S	1	1	2005	E	E
EE-54	1000100011001101000110011	XXXV	A1	IIa	R	1	1	2005	E	E
EE-55	1000100000001101000110011	XXXVI	A2	Ia	S	1	1	2004	S	S
EE-56	0010101001001101000110011	XXXVII	A2	Ia	R	1	1	2004	S	S
EE-52	0010001001001101000110011	XXXVIII	A2	IIa	R	1	1	2005	N	L

^a RG57 fingerprint is denoted using '1' and '0' to indicate presence or absence, respectively, of bands 1-25 recognized by the RG57 probe (Goodwin et al., 1992).

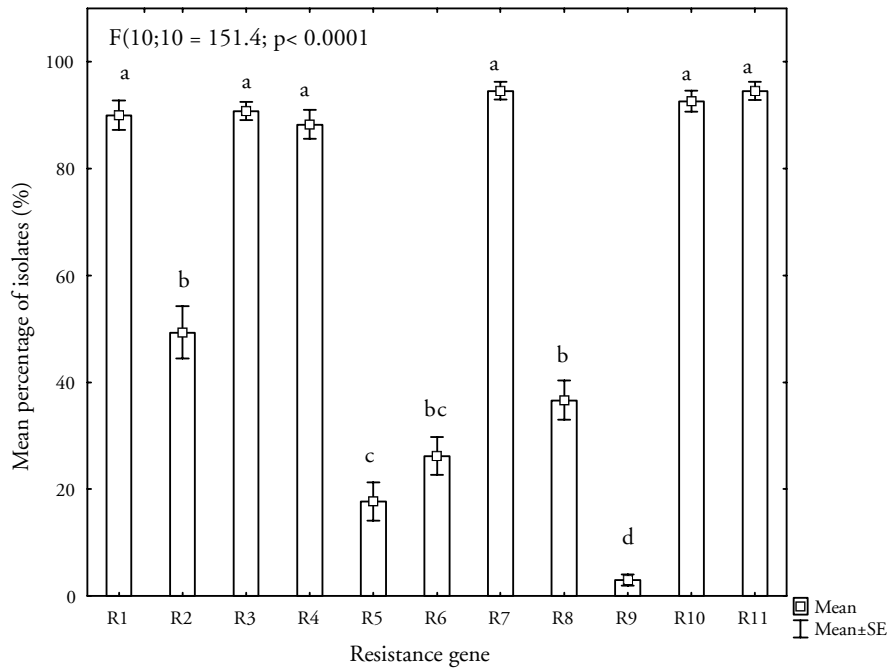
^b RG57 genotypes are numbered with Roman numerals consecutively according to Bakonyi et al. (2002b).

^c S, sensitive; I, intermediate; R, resistant.

^d Regions of Estonia: C, Central; E, East; N, North; SE, South-East; SW, South-West.

^e L, large-scale conventional; E, experimental; S, small-scale conventional production.

Fig. 1. Frequency (%) of virulence to potato R-genes in the Estonian population of *Phytophthora infestans* in 2004-2007.





Runno-Paurson, E., Rimmel, T., Koppel, M., Tähtjärv, T. 2010

OCCURRENCE AND DISTRIBUTION MATING TYPES A1
AND A2 OF *PHYTOPHTHORA INFESTANS*
IN EASTERN ESTONIA.

Agronomy Research (accepted for publication)

Occurrence and distribution mating types A1 and A2 of *Phytophthora infestans* in eastern Estonia

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Abstract. *Phytophthora infestans* (Mont.) de Bary is one of the most serious and economically important pathogens in potato fields worldwide, including Estonia. Under favourable conditions it can destroy the whole potato haulm and cause a considerable yield loss. In Estonia, the average yield loss due to late blight can reach 20-25% and in untreated fields even more. Without control of potato late blight it is not possible to achieve high-quality crop yield. In Estonia, fungicides are used routinely in conventional potato production, but under favourable conditions for the disease, with heavy pressure of the pathogen, protecting large areas is complicated. *P. infestans* isolated from potato leaves and tubers were collected from a region of Eastern Estonia during 2001 to 2007. In total, 133 isolates were assessed for mating type. The average frequencies of mating types A1 and A2 were 59% and 38% respectively. The data indicate that in the Estonian population of *P. Infestans*, both mating types occur simultaneously within fields, with a potential for sexual recombination.

Keywords: mating type, *Phytophthora infestans*, sexual reproduction

Introduction

Late blight is caused by the fungal-like oomycete *P. infestans*, which can reproduce both sexually and asexually. For sexual reproduction, *P. infestans* requires both A1 and A2 mating types to produce gametangia (Fry & Goodwin, 1997). The centre of diversity of this oomycete is located in the highlands of Mexico (Fry & Goodwin, 1997), where both

mating types originate from. At least two different migration events have occurred from Mexico. The first is postulated to have occurred before 1845, after which *P. infestans* swept through Europe and Ireland resulting in the death of over one million people due to starvation, and emigration of 1.5 million people to other parts of Europe or North America (Drenth *et al.*, 1994). The second migration occurred in the 1970s to 1990s, bringing the A2 mating type out of Mexico and also containing genetically diverse and aggressive strains (Fry *et al.*, 1993). In Estonia, the A2 mating type was detected for the first time in 1987 by researchers of The All Union Institute of Plant Pathology (Vorobyeva *et al.*, 1991).

In the present study, phenotypic characteristics of *P. infestans* isolates, collected from potato plants during 2001 to 2007 in eastern Estonia, are reported. The main aim was to detect the temporal changes in population parameters throughout the seven years within a distinct location. It was also observed if the mating type ratio in this area suggests the occurrence of sexual reproduction. The results are compared with the findings of similar studies performed in other European countries in recent years.

Materials and methods

In total, 133 isolates of *Phytophthora infestans* were collected from Estonia during 2001-2007. Most of the isolates originated from leaves and a minority from tubers. The isolates were sampled randomly from a location in eastern Estonia; the procedure was repeated in seven years. All sites used in the seven years were experimental field trial plots at the Jõgeva Plant Breeding Institute (one experimental field per year, labeled here as Jõgeva 2001, Jõgeva 2002, Jõgeva 2003, Jõgeva 2004, Jõgeva 2005, Jõgeva 2006 and Jõgeva 2007). The study area is characterized by high genetic diversity of the host plants including several genotypes that have race specific genes (Runno-Paurson *et al.*, 2009). No fungicides were used for late blight control.

Blighted leaves (one per plant) were collected in the period from the emergence of disease until the end of the growing season in all years. Fourteen to forty leaflets, each with single lesions, were collected from individual plants. Isolations were carried out as described in Runno-Paurson *et al.* (2009).

For mating type determination was used a method as described Runno-Paurson *et al.* (2009). In 2002 and 2003, the determinations were conducted at Cornell University by using a tester strain (US970001 for the A1 mating type and US940480 for the A2 mating type). In all other years, the tester isolates described by Lehtinen *et al.* (2007) were used. 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 among *P. infestans* isolates between years were tested using a logistic analysis (GENMOD procedure in SAS) with a multinomial response variable (A1, A2, or both).

Results and discussion

133 isolates were assessed for mating type. The frequencies of A1 and A2 were 59% and 38% respectively. Self-fertile isolates were also found (3%). There were considerable differences ($\chi^2 = 45.74$, $df = 12$, $p < 0.001$) in the proportion of A1 and A2 between years. A2 mating type was found in all years except in 2002 (Figure 1). The frequency of A2 mating type was quite higher in 2001, 2003, 2004, 2005 and 2007 (41-71%).

The average percentage of A2 mating type in the current study was 38%, which is similar to the results of previous studies in 2002-2003 (Runno-Paurson *et al.*, 2009). There have been reports of a lower A1:A2 ratio from several European countries (Hermansen *et al.*, 2000; Bakonyi *et al.*, 2002a; *et al.*, 2002b; Cooke *et al.*, 2006; Lehtinen, *et al.*, 2007). A higher proportion of A2 mating type has been found in Austria, The Netherlands and Poland (Avendaño Córcoles, 2007; Zwankhuizen *et al.*, 2000; Iiwka *et al.*, 2006).

We found no consistent directional change in the proportions of mating types throughout the study years. However, the prevalence of A2 mating type fluctuated strongly, being almost absent in some years and making up over a half of the population in others (Fig. 1). More precisely, in 2001, 52% of the isolates were A2 mating type, whereas in 2002, A2 mating type was not found. Since then the proportion of A2 mating type increased abruptly from 0% to 44% in 2003 and 61% in 2004. Even though the proportion fell to 3% in 2006, it increased once again to 71% by 2007.

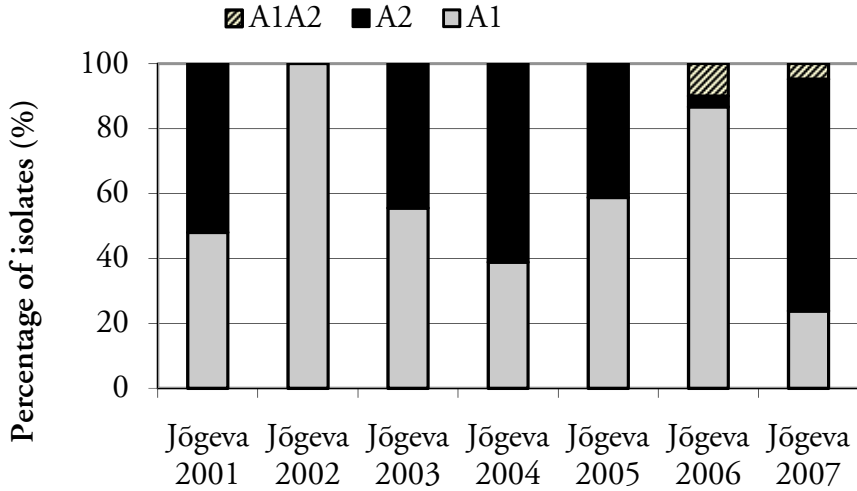


Figure 1. Percentages of mating types among isolates of *Phytophthora infestans* from eastern Estonia during 2001-2007.

In general, it can be noted that in many years the ratio of the two mating types appeared to be close to 50:50, which is the most effective ratio for sexual reproduction. Both mating types were detected in most (six out of seven) of the studied plots. The presence of both mating types in the same field indicates that oospores would be produced in potato foliage (Turkensteen *et al.*, 2000). This changes pathogen epidemiology substantially. Sexual reproduction increases genetic diversity and leads to soil contamination with oospores and associated early late-blight infection. Previous results by Runno-Paurson *et al.* (2009) indicated that blight epidemics could be severe both in large conventional productions and in organic fields. The results of this study confirm the same situation in experimental fields. Therefore, organic farm managers who do not use fungicides are recommended to apply other preventive methods such as crop rotation. This can be important because continuous potato cropping in small scale conventional fields further increases the risk of oospore-derived infections and may cause earlier attacks and consecutive yield loss (Runno-Paurson *et al.*, 2009).

Conclusions

The results of this study indicate that the ratio of *P. infestans* A1:A2 mating types is suitable for sexual reproduction. In most of the studied years,

both mating types were found from the same field plots. The probable occurrence of oospore formation makes it important to apply crop rotation as a preventive measure.

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References

- Avendaño Córcoles, J. 2007. Survey of *Phytophthora infestans* population in Austria based on phenotypic and molecular markers. Doctoral Thesis. University of Natural Resources and Applied Life Sciences, Vienna, Austria. Department of Biotechnology, Institute of Applied Microbiology 138 p.
- Bakonyi, J., Heremans, B. & Jamart, G. 2002a. Characterization of *Phytophthora infestans* isolates collected from potato in Flanders, Belgium. *Phytopathology* **150**, 512-516.
- Bakonyi, J., Ládai, M., Dula, T. & Érsek, T. 2002b. Characterisation of isolates of *Phytophthora infestans* from Hungary. *European Journal of Plant Pathology* **108**, 139-146.
- Cooke, L. R., Carlisle, D. J., Donaghy, C., Quinn, M., Perez, F. M. & Deahl, K L. 2006. The Northern Ireland *Phytophthora infestans* population 1998-2002 characterized by genotypic and phenotypic markers. *Plant Pathology* **55**, 320-330.
- Drenth, A., Tas, I. C. Q. & Govers, F. 1994. DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. *European Journal of Plant Pathology* **100**, 97-107.
- Fry, W. E. & Goodwin, S. B. 1997. Re-emergence of potato and tomato late blight in the United States. *Plant Disease* **81**, 1349-1357.

- Hermansen, A., Hannukkala, A., Hafskjold Naerstad, R. & Brurberg, M. 2000. Variation in populations of *Phytophthora infestans* in Finland and Norway: mating type, metalaxyl resistance and virulence phenotype. *Plant Pathology* **49**, 11-22.
- Lehtinen, A., Hannukkala, A., Rantanen, T. & Jauhiainen, L. (2007). Phenotypic and genetic variation in Finnish potato-late blight populations, 1997-2000. *Plant Pathology* **56**, 480-491.
- Runno-Paurson, E., Fry, W. E., Myers, K. L., Koppel, M. & Mänd, M. 2009. Characterization of *Phytophthora infestans* isolates collected from potato in Estonia during 2002-2003. *European Journal of Plant Pathology* **124**, 565-575.
- Śliwka, J., Sobkowiak, S., Lebecka, R., Avendao-Cóles, J. & Zimnoch-Guzowska, E. 2006. Mating type, virulence, aggressiveness and metalaxyl resistance of isolates of *Phytophthora infestans* in Poland. *Potato Research* **49** (3), 155-166.
- Turkensteen, L. J., Flier, W. G., Wanningen, R. & Mulder, A. 2000. Production, survival and infectivity of oospores of *Phytophthora infestans*. *Plant Pathology* **49**, 688-696.
- Vorobyeva, Yu. V., Gridnev, V. V., Bashaeva, E. G., Pospelova, L. A., Kvasnyuk, N. Ya., Kuznetsova, L. N., Shemyakina, V. P., Morozova, E. V., Zherebtsova, L. N. & Rozalyeva, V. V. 1991. On the occurrence of the A2 mating type isolates of *Phytophthora infestans* (Mont.) d by in the USSR. *Mikologija i fitopatologija* pp. 62-67.
- Zwankhuizen, M. J., Govers, F. & Zadoks, J. C. 2000. Inoculum sources and genotypic diversity of *Phytophthora infestans* in Southern Flevoland, the Netherlands. *European Journal of Plant Pathology* **106**, 667-680.



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DO CROP MANAGEMENT PRACTICES INFLUENCE
THE STRUCTURE OF *PHYTOPHTHORA INFESTANS*
POPULATION?

European Journal of Plant Pathology (in review)

Do crop management practices influence the structure of *Phytophthora infestans* population?

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Abstract

A total of 196 isolates of *Phytophthora infestans* were collected from conventional and organic productions in northern Estonia at several potato fields during 2004-2005. Most of the isolates were tested for mating type, virulence, metalaxyl resistance and a sub-group for mtDNA haplotype. In Estonia, 41% of the 175 isolates tested were A2 mating type. All 11 virulence factors were found among the tested isolates. The mean number of virulence factors per isolate was 6.6, with a very low frequency of virulence against resistance gene R9 (2%). The most common race was 1.3.4.7.10.11, representing altogether almost a half (49%) of the studied strains. Three mitochondrial DNA haplotypes were found (Ia, IIa and IIb). Haplotype Ia was prevalent (74%) among 66 isolates. The results indicated several differences between cropping systems in the population structure of *P. infestans*. Diversity of combined multilocus genotype measured by the normalized Shannon diversity index was very high in large scale conventional fields (0.97), while the overall value was 0.64. Complex races were found to be typical for organic farms, and there was also a higher extent of A2 mating type in organic fields than

in other potato productions. Resistance to metalaxyl was most common in large scale conventional fields. Such differences can have important implications for determining the optimal strategies in potato late blight management.

Introduction

Potato late blight, caused by the oomycete *Phytophthora infestans*, is one of the most devastating diseases of potato worldwide and is an ongoing threat to potato growers in temperate regions, requiring vigilance and often numerous applications of fungicide for effective control (Cooke et al., 2003). Under favourable conditions the pathogen not only reduces yield by destroys foliage and prevents the growth of tubers, it also promotes rotting of the tubers before and during storage (Smart & Fry, 2001) and thereby causes a considerable yield loss. In Estonia it is not possible to achieve high yield with good quality in conventional productions without control against the late blight pathogen (Koppel, 1997). In organic fields, where mostly varieties with high resistance are used the yield loss may reach up to 50% (Runno-Paurson *et al.*, unpublished data). Protective copper based fungicides, which are used in organic production systems in Europe are prohibited in Estonia.

Before the 1970s, European populations of the late blight pathogen appear to have consisted solely of a single clonal lineage of the A1 mating type, known as US-1, which has the mitochondrial DNA (mtDNA) haplotype Ib (Goodwin et al., 1994). In recent years, in most European populations the 'old' population genotypes have not been detected. Unlike the former populations, which were made up exclusively of isolates belonging to the A1 mating type, 'new' genotypes comprised isolates of both mating types (Spielman et al., 1991; Day & Shattock, 1997; Lebreton & Andrivon, 1998), with the potential to reproduce sexually. The new population also contains both Ia and IIa mtDNA haplotypes (Day & Shattock, 1997; Lebreton & Andrivon, 1998). Oospores can withstand unfavourable conditions and survive in the soil, thus affecting the epidemiology of the disease (Mayton et al., 2000). In many cases, there has been an increase in the complexity of virulence phenotypes (Sujkowski et al., 1996; Hermansen et al., 2000, Lehtinen et al., 2008).

P. infestans reproduces sexually in most European countries (Zwankhuizen et al., 2000; Bagirova & Dyakov, 1998; Schöber-Butin, 1999; Anderson et al., 1998; Brurberg et al., 1999; Lehtinen et al., 2006; Lehtinen et al., 2008; Avendaño Córcoles, 2007; Iiwka et al., 2006). The proportion of A2 mating type isolates collected from commercial potato fields has remained low in the France, Germany, Belgium and Switzerland (Gisi & Cohen, 1996; Bakony et al., 2002a), whereas in the Netherlands, Nordic countries and UK it has reached over 50% (Hermansen et al., 2000; Turkensteen et al., 2000; Lehtinen et al., 2006; Lehtinen et al., 2008, Lees et al., 2009).

In Estonia, the A2 mating type was first found in 1987. The data from the period between 2002-2003 indicated the presence of both mating types together in most of the studied sites, suggesting the occurrence of sexual reproduction in Estonian populations (Runno-Paurson et al., 2009). In such a situation, management of the new sexually reproducing populations is a challenge for conventional production and can be crucial for the economy of organic potato producers (Hannukala & Lehtinen, 2005).

The number of organic farms has increased since early 1990s and risen notably since 2002. About 10 percent of all cultivable land is used for potato production. However, the organic farms in Estonia have various backgrounds, for example, many of them do not rotate crops and seed potatoes are often not certified. This and perhaps more importantly the prohibition to use fungicides in these farms allows one to expect that organic farms may have a higher risk of late blight epidemics and consequent yield loss than do conventional fields.

The main objective of this study was to reveal the population structure of *P. infestans* in organic and conventional productions in Estonia to find possible differences between cropping systems. It was asked if organic productions had more diverse and/or more resistant populations than conventional fields, which would, in turn, pose a higher risk of yield loss. The results of this study can be compared with the populations in other regions of Estonia and other European countries to get a larger picture of the spatiotemporal variation in the population structure of this pathogen.

Materials and methods

Collection and isolation of isolates

In two consecutive years, 2004 and 2005, 196 isolates of *P. infestans* were collected from twelve potato fields (4 organic, 4 small scale conventional and 4 large scale conventional productions) in northern Estonia (Table 1). The small and large scale conventional farms used in this study differed in their usage of agrotechnical methods. In particular, in the small scale conventional farms, farmers used seed potatoes of uncertain quality and did not follow the crop rotation. Chemical late blight treatment was applied only once per growing season. In the large scale conventional farms, farmers used high-quality certified seed potatoes, adhered to the official crop rotation requirements and made at least 6-7 treatments against potato late blight per season. Copper based fungicides are not used in Estonian organic productions.

Nine to twenty-three leaflets, each with single lesion (one per plant) were collected in organic and small scale farms twice in both years: at the beginning of outbreak and in the end of the growing season (an approximately equal number of isolates was taken early and late in the season). In early stages of the epidemics, approximately 20-25% of leaf area of the infected plants and less than 10% of plants were infected with late blight. In later stages, about 20-40% of leaf area and more than 50% of the plants were infected. In large scale farms, samples were collected at the beginning of outbreak. The plants were selected by randomising the distance from field edges, and from each plant the blighted leaf was also randomly chosen, excluding those that had several or no lesions.

Isolations were carried out by placing a fragment of infected leaf tissue between ethanol and flame-sterilized tuber slices. Tubers of susceptible cultivars without known R genes were used (Berber or Bintje). The slices were put into sterile Petri dishes with a moist filter paper disk on top. The Petri dishes were incubated for 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 & 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. Mitochondrial DNA haplotype analyses were done in November-December 2005.

Phenotypic analyses

Mating types were determined by the method described in Runno-Paurson *et al.* (2009). Observed oospore formation was interpreted as the occurrence of self-fertility of the isolates. The tester isolates were the same as those described in Lehtinen *et al.* (2007). The resistance to metalaxyl of all 412 isolates was tested using a modification of the floating leaflet method (Hermansen *et al.*, 2000) as described in Runno-Paurson *et al.* (2009). The specific virulence of each of the 432 isolates was determined by using Black's differential set of potato genotypes containing resistance genes R1-R11 (Malcolmson & Black, 1966) (provided by Scottish Agricultural Science Agency). Laboratory procedures were performed as described in Runno-Paurson *et al.* (2009).

Neutral marker assessment

For determining the mitochondrial DNA (mtDNA) haplotype, from each field at least four isolates were selected (66 in total). The isolates were selected so that the proportion of mating types was approximately the same as in the main sample of the particular field. The mtDNA haplotype of the isolates in the subset was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) with method by Griffith & Shaw (1998). The mtDNA haplotype detection was conducted in MTT Agrifood Research Finland. Isolates were transplanted into 10 cm Petri dishes on pea agar and incubated until the surface of the agar was filled with mycelia. Then DNA was extracted and purified using Dneasy kit (Quiagen). The approximate DNA concentration in water solution was determined by comparing the fluorescence of the solution and standard solutions on agar plate containing ethidium bromide under UV light. DNA concentration 1ng/μl or more in PCR mix was needed to get clear PCR product band in following electrophoresis. The DNA polymerase DyNAzyme II (Finnzymes) was used, concentration in PCR mix was 0.02 U/μl. The primers used for amplification of mt DNA regions P2 and P4 were F2 (5'-TTCCCTTTGTCCTCTACCGAT-3')+ R2 (5'-TTACGGCGGTTTATGCACATACA-3') and F4 (5'-TGGTCATCCAGAGGTTTATGTT-3')+ R4 (5'-CCGATACCGATAACCAGACCAA-3'), 0.2 p mol/μl. The PCR program used for amplification of P2 was 1x 90°C for 5min, 35x (94°C for 30 s, 64°C for 1 min, 72°C for 1 min), 1x (72°C for 5 min, 11°C for 30s and 4°C for ever). In case of P4, the program was similar, but 55°C annealing temperature was used. Digestions with MspI (P2) and EcoRI (P4) were

performed at 37°C overnight. Then the digested DNA samples were loaded into 1% agarose gel containing ethidium bromide. After run, the restriction patterns were visualized using UV transilluminator.

Data analysis

Forty-six isolates were characterized as multilocus genotypes consisting of mating type, mtDNA haplotype and virulence phenotype. Genotypic diversity as well as race complexity were calculated as normalized Shannon diversity indices (Sheldon, 1969): $H_s = -\sum g_i \ln g_i / \ln N$, where g_i is the frequency of the i th multilocus genotype and N is the sample size. The normalized index ranges from 0 (there is no diversity) to 1 (each isolate represents a unique genotype).

Statistical analyses were performed with SAS/STAT version 9.1 (SAS Institute Inc., Cary, NC, USA) using the GENMOD procedure. Logistic analyses were used to test for the dependence of mating type (multinomial response variable: A1, A2 or both) and haplotype (binomial: Ia vs. IIa) on locations (twelve fields) and years (2004 vs. 2005). Similar analyses were performed to compare the proportions of different mating types, haplotypes and isolates resistant to metalaxyl between cropping systems (small and large scale conventional fields and organic fields), i.e. all studied fields were assigned to one of these three groups.

Separate logistic analyses were used to test for the difference in the prevalence of virulence against different R genes (virulent vs. non-virulent) between years, the dependence of mating type on haplotype and race prevalence (unique vs. prevalent), and the association between virulence complexity (average number of R-genes overcome) and resistance to metalaxyl. The dependence of virulence complexity on cropping system was analysed with one-way ANOVA, as were the differences in the Shannon index values between cropping systems.

Results

Mating type determination

Among the 175 tested isolates, 57% were A1 mating type, 41% were A2 mating type and 2% were self-fertile. At 11 of the 12 fields, both A1 and

A2 mating types were detected. The proportion of the A2 mating type increased abruptly from 28% in 2004 to 54% in 2005 ($\chi^2 = 11.87$, d.f. = 1, $p = 0.0006$). There were further differences between cropping systems ($\chi^2 = 9.60$, d.f. = 2, $p = 0.0082$), the proportion of A2 being highest in organic fields and lowest in large scale conventional fields (Table 2).

Resistance to metalaxyl

In total, 110 isolates were screened for resistance to metalaxyl. In the two years, 49% of the isolates were resistant to metalaxyl, 34% were intermediate and 17% were classified as sensitive. Of the metalaxyl resistant strains, 65% were A1 mating type, 30% were A2 mating type and 5% were self-fertile; however, the association between metalaxyl resistance and mating type was not significant ($\chi^2 = 3$, d.f. = 1, $p = 0.083$).

Considerable differences between potato cropping systems were observed ($\chi^2 = 23.75$, d.f. = 2, $p < 0.0001$). In particular, in large scale conventional fields, 66% of tested isolates were resistant to metalaxyl, while in small scale farm fields 26% and in organic fields only 14% of the isolates were resistant (Table 3). There were no differences between the collecting years (2004 vs 2005, $\chi^2 = 0.98$, d.f. = 1, $p = 0.42$); however, when compared to the data collected in 2002-2003 (Runno-Paurson et al., 2009) the prevalence of metalaxyl resistant isolates had increased from 30 to 49% ($\chi^2 = 5.45$, d.f. = 1, $p = 0.02$).

Virulence

All known virulence factors (to overcome genes R1-R11) were found among the 196 isolates. Nearly all isolates were virulent on differentials with genotypes R1, R3, R4, R7, R10 and R11. Virulence factor 9 (1%) was rare and factors 5 (10%) and 8 (10%) were relatively rare (Fig 1, Table 4). During the two year period, an increase in virulence factors 2, 5, 8 and 9 was noticed (factor 2: $\chi^2 = 10.95$, d.f. = 1, $p = 0.0009$; factor 5: $\chi^2 = 9.38$, d.f. = 1, $p = 0.0022$; factor 8: $\chi^2 = 16.03$, d.f. = 1, $p < 0.0001$; factor 9: $\chi^2 = 5.55$, d.f. = 1, $p = 0.019$). After applying a Bonferroni correction (since eleven comparisons were made), only the differences in virulence factors 2, 5 and 8 remain significant. Thirty-eight races were detected (Table 5). The two most common races made up 70% (Table 5) of the isolates tested. The overall virulence complexity (average number of R-genes overcome) was 6.7 (Table 5). Virulence complexity was the

highest in organic farms (7.3). Complex races predominated in organic fields, but were less common in small and large scale conventional fields ($F_{(193)} = 8.49$, $p = 0.00029$). The overall normalized Shannon diversity index was 0.38 and differed significantly between cropping systems ($F_{(2)} = 23.89$, $p = 0.0028$). This index was as high as 0.71 in large scale conventional fields, but much lower in small scale (0.13) and organic fields (0.18).

Mitochondrial DNA haplotype

Three mitochondrial haplotypes (Ia, IIa and IIb) were detected among 66 isolates tested. Two isolates of haplotype IIb were found from large scale conventional fields. The majority of isolates were haplotype IIa (74%) and minority were Ia (23%). No significant differences were found in the frequencies of Ia and IIa between years ($\chi^2 = 1.46$, d.f. = 1, $p = 0.23$). However, differences between cropping systems were observed ($\chi^2 = 8.38$, d.f. = 2, $p = 0.015$), with the highest proportion of IIa in large scale conventional fields and lowest in organic fields. Interestingly, in the latter, only one haplotype (Ia) was detected (Table 6). There was no association between mating type and haplotype ($\chi^2 = 0.76$, d.f. = 1, $p = 0.38$). There were no differences between isolates taken early *vs* late during the season in the prevalence of different haplotypes; nor in the proportions of mating types, metalaxyl resistance or the number of R-genes overcome (statistics not shown).

Multilocus genotypes

Eighteen multilocus genotypes were identified among 46 isolates. In large scale conventional fields, most of the isolates were unique, i.e. found only once (Table 7). All of these belonged to the 'new' population of the pathogen that displaced the US-1 clonal lineage (A1 mating type, Ib mtDNA haplotype) (Spielman *et al.*, 1991; Leberton & Andrivon, 1998). Genotypic diversity measured by the normalized Shannon index was 0.64 and differed significantly between cropping systems ($F_{(2)} = 41.76$, $p < 0.001$). In large scale conventional fields this index was 0.97, in small scale fields 0.53 and in organic fields only 0.28.

Discussion

The results of this study suggest that there may be considerable differences between potato cropping systems in various aspects of the population structure of *P. infestans* inhabiting the fields. However, it is probable that different management practices, mainly fungicide use but also crop rotation and the source of potato seeds, rather than the cropping system *per se*, are behind these differences. Dissimilarities were found in the prevalence of mating types, virulence genes, mtDNA haplotypes and resistance to metalaxyl. These figures were also not always similar to those found in previous studies in this or other geographical regions, implying a noticeable spatial and temporal variation in *P. infestans* population parameters.

Nevertheless, the average proportion of A2 mating type found in this study (41%) was consistent with the results of a previous study conducted in Estonia (Runno-Paurson et al., 2009). Somewhat lower prevalence of the A2 mating type has been reported in Belgium (Bakonyi et al., 2002 a), Finland (Hermansen et al., 2000; Lehtinen et al., 2007; Lehtinen et al., 2008) and Hungary (Nagy et al., 2006). Meanwhile, a higher proportion of A2 mating type has been detected in certain years in Austria (Avendao Córcoles, 2007), Czech Republic (Mazáková et al., 2006), Finland (Lehtinen et al., 2007), Denmark and Sweden (Lehtinen et al., 2008), Hungary (Bakonyi et al., 2002b), Poland (Iiwka et al., 2006) and The Netherlands (Zwankhuizen et al., 2000).

The presence of both mating types in the same field indicates the possibility of oospore production in potato foliage (Turkensteen et al., 2000). In this study, both mating types were detected in nearly all sites, with a single exception of an organic field in 2004. Different results have been reported from Finland (Lehtinen et al., 2007), Netherlands (Zwankhuizen et al., 2000) and Scotland (Cooke et al., 2003), where the co-occurrence of both mating types was more common in organic fields. Based on our results, differences in the A1/A2 ratio between cropping systems can be suggested, even though larger sample sizes are needed to explicitly prove this finding. For instance, in organic fields, 62% of isolates were A2 mating type whereas in large scale conventional farm fields only 31% of isolates were A2 mating type (Table 2). The possibly higher prevalence of A2 mating type, both mating types found from most fields, and no rotation may presume higher risk for sexual reproduction at organic fields compared to other cropping systems.

Further differences between cropping systems were evident in the resistance of isolates to metalaxyl fungicides. Metalaxyl resistant isolates were found four times more often (Table 3) in large scale conventional fields than in organic fields. Such difference could be explicable with the usage of metalaxyl products in large scale conventional fields, even though no significant differences were detected between large scale conventional fields treated and untreated with metalaxyl (statistics not shown). Furthermore, it is possible that the overall prevalence of metalaxyl resistance seems to have increased in recent years: in a previous study (Runno-Paurson et al. 2009), the average percentage of resistant isolates was found to be 30%, but in the present study, this figure had increased to 49%. In both years, but especially in 2004, the epidemics started earlier and were more severe than those observed in 2002-2003 by Runno-Paurson et al. (2009). The reason for such increase may have been the intensive use of metalaxyl in Estonia against the heavy late blight pressure during those years; however, we cannot be certain that this difference is not coincidental.

The Estonian population of *P. infestans* is most similar in the frequency of virulence factors to those described recently in Finland and Norway (Hermansen et al., 2000; Lehtinen et al., 2006), and France and Switzerland (Leberton & Andrivon, 1998; Knapova & Gisi, 2002; Pilet et al., 2005). More diverse and complex populations have been reported from Russia in 1997-1998 (Elansky et al., 2001) and Poland in 2002-2004 and 2005-2006 (Iiwka et al., 2006; Lebecka et al., 2007). The race found to be most common in this study (1.3.4.7.10.11) was also prevalent in most European populations (Andrivon, 1994; Leberton & Andrivon, 1998; Zimnoch-Guzowska, 1999; Hermansen et al., 2000; Knapova & Gisi, 2002; Lehtinen et al., 2007; Lehtinen et al., 2008). In the current study, two of the most common races made up 70% of the tested isolates. This is quite different from the results of the previous study in 2002-2003, where nearly half (48%) of the races were detected only once (Runno-Paurson et al., 2009). The mean number of virulence genes per isolate in this study was 6.1 in 2004, and increased to 7.1 by 2005. The mean value found in Estonia (6.6) is higher than those found in Norway (5.8, Hermansen et al., 2000), Finland in 1990-1996 (5.3, Hermansen et al., 2000) and 1997-2000 (6.0, Lehtinen, et al., 2007), Birobijan region in Russia (5.5, Elansky et al., 2001), Northern China (5.1, Guo et al., 2009) Netherlands (4.7, Schöber & Turkensteen, 1992) and France (4.7, Leberton & Andrivon, 1998), but it is approximately at the same

level as this found in Denmark (6.92) and Sweden (6.87) (Lehtinen et al., 2008) in 2003. Higher values have been found in Poland (7.8) (Iiwka et al., 2006), France and Switzerland (7.6) (Knapova & Gisi, 2002), and Russia (Sakhalin 10, Ekaterinburg 8.9, Irkutsk 8.4, Vladivostok 8.3, Khabarovsk 8.3 and Moscow region 8.1) (Elansky et al., 2001).

Race diversity calculated by the normalized Shannon diversity index showed a much lower value (0.38) in this study compared to the very high diversity among isolates collected from Estonia in 2002 to 2003 (0.89, Runno-Paurson, et al. 2009). The results of the current study were more similar to those found in Austria (Avenidao Córcoles, 2007) and Finland and Norway (Hermansen et al., 2000). The diversity index was much higher among isolates collected from large scale conventional fields (0.71) than from organic (0.18) and small scale conventional fields (0.13). This result is particularly puzzling because, unlike smaller farms, large scale farms used certified potato seed tubers and applied rotation. Somewhat contrainituitively, the most complex races were actually more common in organic fields.

Similarly, the multilocus genotypic diversity was found to be particularly high in large scale conventional productions. A possible explanation could be related to the background of seed potatoes used in different farms. In particular, large scale conventional producers import seed potatoes directly from Central Europe (Germany and Denmark), after which the tubers are multiplied locally for two years. Small scale conventional and organic producers, on the other hand, use seed potatoes that originate from Estonian seed potato growers and are likely to contain only the locally prevalent pathogen genotypes.

Another dissimilarity found between cropping systems was the higher prevalence of the generally less common IIa haplotype in large scale conventional fields compared to other field types. The reason for such difference may again be the use of imported seed potatoes by large scale farmers. The high proportion of Ia haplotype (74%) in this study differs from the results of the previous study conducted in Estonia (46%, Runno-Paurson et al., 2009). A higher proportion of Ia haplotype has also been observed in Poland, England, Scotland, Wales, The Netherlands and France (Leberton & Andrivon et al., 1998; Cook et al., 2003; Lebecka et al., 2007). Haplotype IIb was found for the first time in Estonia. The Ib haplotype, associated with the old clonal *P. infestans* populations

present in Europe during most of the 20th century (Spielman et al., 1991), was not found.

The used markers were chosen to show mainly phenotypic variability, with genetic variation characterized by mtDNA haplotypes. In further studies it would also be informative to use microsatellite markers to detect the specific relationships between phenotypic and genotypic variation, as reported in Guo et al. (2009).

In conclusion, the results of this study clearly suggest that there may be cropping system-specific differences in the population structure of *P. infestans*, which most probably arise from different management practices in these systems. Such differences can likely lead to variation in the risk of yield loss. In contrast to the previous assumptions, several aspects of the pathogen diversity, such as genotypic diversity, race complexity and the diversity of mtDNA haplotypes appeared to be highest in the large scale conventional fields. On the other hand, the proportion of the novel A2 mating type and virulence complexity were highest in the organic fields. The prevalence of metalaxyl resistance was also highest in the large scale conventional fields. Such differences should not be ignored by producers, and different precautions can be suggested for managing different types of farms. In particular, the conventional farmers may benefit from the use of other control methods beside metalaxyl fungicides to limit the spread of resistance in the pathogen population. However, it would certainly be desirable to repeat these comparisons in further studies incorporating a larger number of fields to confirm more rigorously the differences between management practices. The spatiotemporal variation observed in *P. infestans* population parameters across Europe may imply that managers also need to consider the regional situation to make optimal decisions.

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References

- Anderson, B., Sandström, M., & Strömberg, A. (1998). Indication of soil-borne inoculum of *Phytophthora infestans*. *Potato Research*, *41*, 305-310.
- Andriveau D. (1994). Races of *Phytophthora infestans* in France, 1991-1993. *Potato Research*, *37*, 279-286.
- Avendaño Córcoles J. (2007). *Survey of Phytophthora infestans population in Austria based on phenotypic and molecular markers*. Vienna, Austria: University of Natural Resources and Applied Life Sciences, PhD thesis.
- Bagirova, S. F., Dyakov, & Yu. T. (1998). Participation of *Phytophthora infestans* oospores in spring epidemics resumption. *Sel'skohozyaistvennaya Biologia*, *3*, 69-71.
- Bakonyi, J., Heremans, B., & Jamart, G. (2002 a). Characterization of *Phytophthora infestans* isolates collected from potato in Flanders, Belgium. *Phytopathology* *150*, 512-516.
- Bakonyi, J., Ládai, M., Dula, T., & Érsek, T. (2002 b). Characterisation of isolates of *Phytophthora infestans* from Hungary. *European Journal of Plant Pathology*, *108*, 139-146.
- Brurberg, M. B., Hannukala, & A., Hermansen, A. (1999). Genetic variability of *Phytophthora infestans* in Norway and Finland as revealed by mating type and fingerprint probe RG57. *Mycological Research*, *12*, 1609-1615.
- Caten, C. E., & Jinks, J. L. (1968). Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. *Canadian Journal of Botany*, *46*, 329-348.
- Cooke, D. E. L., Young, V., Birch, P.R.J., Toth, R., Gourlay, F., Day, J.P., Carnegie, S. F., & Duncan, J. M. (2003). Phenotypic and genotypic diversity of *Phytophthora infestans* populations in Scotland (1995-97). *Plant Pathology*, *52*, 181-192.

- Cooke, L., Carlisle, D. J., Donaghy, C., Quinn, M., Perez, F. M., & Deahl, K. L. (2006). The Northern Ireland *Phytophthora infestans* population 1998-2002 characterized by genotypic and phenotypic markers. *Plant Pathology*, *55*, 320-330.
- Day, J. P., Wattier, R. A. M., Shaw, D. S., & Shattock, R. C. (2004). Phenotypic and genotypic diversity in *Phytophthora infestans* on potato in Great Britain, 1995-98. *Plant Pathology*, *53*, 303-315.
- Elansky, S., Smirnov, A., Dyakov, Y., Dolgova, A., Filippov, A., Kozlovski, B., Kozlovskaja, I., Russo, P., Smart, C., & Fry, W. E. (2001). Genotypic analysis of Russian isolates of *Phytophthora infestans* from the Moscow region, Siberia and Far East. *Phytopathology*, *149*, 605-611.
- Gisi, U., & Cohen, Y. (1996). Resistance to phenylamide fungicides: A case study with *Phytophthora infestans* involving mating type and race structure. *Annual Review of Phytopathology*, *34*, 549-572.
- Goodwin, S. B., Cohen, B. A., & Fry, W. E. (1994). Planglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Sciences, USA*, *91*, 11591-11595.
- Griffith, G. W., & Shaw, D. S. (1998). Polymorphisms in *Phytophthora infestans*: Four mitochondrial haplotypes are detected after PSR amplification of DNA from pure cultures or from host tissue. *Applied and Environmental Microbiology*, *64*: 4007-4014.
- Guo, J., van der Lee, T., Qu, D. Y., Yao, Y. Q., Gong, X. F., Liang, D. L., Xie, K. Y., Wang, X. W., & Govers, F. (2009). *Phytophthora infestans* isolates from Northern China show high virulence diversity but low genotypic diversity. *Plant Biology*, *11*, 57-67.
- Hannukkala, A., & Lehtinen A. (2005). Management of potato late blight, *Phytophthora infestans*, inorganic production. In: E. Ritter, A. Carrascal (eds.). 16th triennial conference of the EAPR, Bilbao, Spain.
- Hermansen, A., Hannukkala, A., Hafskjold Naerstad, R., & Brurberg, M. B. (2000). Variation in populations of *Phytophthora infestans* in Finland and Norway: mating type, metalaxyl resistance and virulence phenotype. *Plant Pathology* *49*: 11-22.
- Koppel, M. (1997). Changes in late blight resistance of potato varieties in 1922-1991. In /Problems in contemporary ecology. Temporal changes in the wildlife and environment of Estonia/. Tartu, 97-101.

- Knapova, G., & Gisi, U. (2002). Phenotypic and genotypic structure of *Phytophthora infestans* populations on potato and tomato in France and Switzerland. *Plant Pathology*, *51*, 641-653.
- Lebecka, R., Śliwka, J., Sobkowiak, S., & Zimnoch-Guzowska, E. (2007). *Phytophthora infestans* population in Poland. *PPO-Special Report no. 12*, 155-159.
- Lebreton, L., & Andrivon, D. (1998). French isolates of *Phytophthora infestans* from potato and tomato differ in phenotype and genotype. *European Journal of Plant Pathology*, *104*, 583-594.
- Lees, A. K., Cooke, D. E. L., Stewart, J. A., Sullivan, L., Williams, N. A., & Carnegie, S. F. (2009). *Phytophthora infestans* population changes: implications. *PPO-Special Report no. 13*, 55-60.
- Lehtinen, A., Hannukkala, A., Andersson, B., Hermansen, A., Le, V. H., Naerstad, R., Brurberg, M. B., Nielsen, B. J., Hansen, J. G., & Yuen, J. (2008). Phenotypic variation in Nordic populations of *Phytophthora infestans* in 2003. *Plant Pathology*, *57*, 227-234.
- Lehtinen, A., Hannukkala, A., Rantanen, T., & Jauhiainen, L. (2007). Phenotypic and genetic variation in Finnish potato-late blight populations, 1997-2000. *Plant Pathology*, *56*, 480-491.
- Malcolmson, J. F., & Black, W. (1966). New R genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica*, *15*, 199-203.
- Mazáková, J., Táborský, V., Zouhar, M., Rysánek, P., Hausvater, E., & Dolezal, P. (2006). Occurrence and distribution of mating types A1 and A2 of *Phytophthora infestans* (Mont.) de Bary in the Czech Republic. *Plant Protection Science*, *42*, 41-48.
- Mayton, H., Smart, C. D., Moravec, B. C., Mizubuti, E. S. G., Muldoon, A. E., & Fry, W. E. (2000). Oospore survival and pathogenicity of single oospore recombinant progeny from a cross involving US-17 and US-8 genotypes of *Phytophthora infestans*. *Plant Disease*, *84*, 1190-1196.
- Nagy, Z. Á., Bakonyi, J., Som, V., & Érsek T. (2006). Genetic Diversity of the population of *Phytophthora infestans* in Hungary. *Acta Phytopathologica et Entomologica Hungarica*, *41*, 53-67.
- Pilet, F., Pelle, R., Ellisseche, D., & Andrivon, D. (2005). Efficacy of the R2 resistance gene as a component for the durable management of potato late blight in France. *Plant Pathology*, *54*, 723-732.

- Runno-Paurson, E., Fry, W. E., Myers, K. L., Koppel, M., & Mänd, M. (2009). Characterization of *Phytophthora infestans* isolates collected from potato in Estonia during 2002-2003. *European Journal of Plant Pathology*, 124, 565-575.
- Schöber-Butin, B. (1999). *Phytophthora infestans*: pathotypes, mating types and fungicide resistance in Germany. PAV-Special Report Number 5, pp. 178-182.
- Sheldon, A. L. (1969) Equitability indices: Dependence on the species count. *Ecology*, 50, 466-467.
- Shattock, R. C., Tooley, P. W., & Fry, W. E., 1986. Genetics of *Phytophthora infestans*: Determination of recombination, segregation, and selfing by isozyme analysis. *Phytopathology*, 76, 410-413.
- Śliwka, J., Sobkowiak, S., Lebecka, R., Avendao Córcoles, J., & Zimnoch-Guzowska, E. (2006). Mating type, virulence, aggressiveness and metalaxyl resistance of isolates of *Phytophthora infestans* in Poland. *Potato Research*, 49 (3), 155-166.
- Smart, C. D., & Fry, W. E. (2001). Invasions by the late blight pathogen: renewed sex and enhanced fitness. *Biological Invasions* 3, 235-243.
- Spielman, L. J., Drenth, A., Davidse, L. C., Sujkowski, L. J., Gu, W., Tooley, P. W., & Fry, W. E. (1991). A second world-wide migration and population displacement of *Phytophthora infestans*? *Plant Pathology*, 40, 422-430.
- Sujkowski, L. S., Goodwin, S. B., Fry, & W. E. (1996). Changes in specific virulence in Polish populations of *Phytophthora infestans*: 1985-91. *European Journal of Plant Pathology*, 102, 555-561.
- Turkensteen, L. J., Flier, W. G., Wanningen, R., & Mulder, A. (2000). Production, survival and infectivity of oospores of *Phytophthora infestans*. *Plant Pathology* 49: 688-696.
- Zimnoch-Guzowska, E. (1999). Late blight and blight research in Central and Eastern Europe. In: *Proceedings of the Global Initiative on Late Blight Conference, Late Blight: A Threat to Global Food Security*, Vol 1, pp. 9-14.
- Zwankhuizen, M. J., Govers, F., & Zadoks, J. C. (2000). Inoculum sources and genotypic diversity of *Phytophthora infestans* in Southern Flevoland, the Netherlands. *European Journal of Plant Pathology*, 106, 667-680.

Legends

Figure 1. Frequency (percentage) of virulence to potato R-genes among isolates of *Phytophthora infestans* collected from different cropping systems in Estonian (2004-2005).

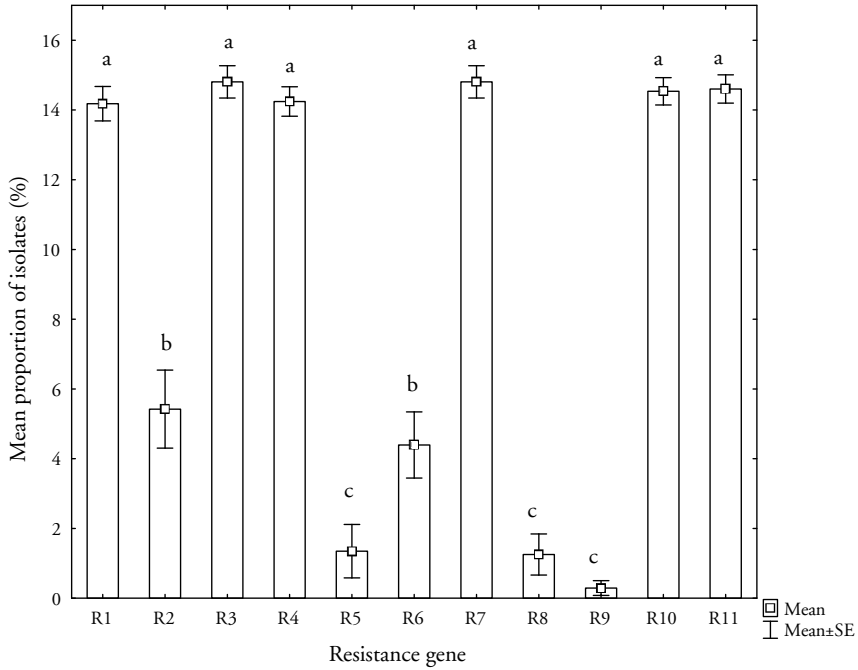


Table 1. Sampling of *Phytophthora infestans* isolates collected from different cropping systems in Estonia (2004-2005)

Cropping system	Tested for		
	Mating type (n)	Virulence (n)	mtDNA haplotype (n)
Organic	42	54	24
Small scale conventional	61	68	24
Large scale conventional	72	74	18
Total	175	196	66

Table 2. Percentages of mating types among isolates of *Phytophthora infestans* from different cropping systems in Estonia (2004-2005)

Cropping system	A1 (%)	A2 (%)	A1A2 (%)	Isolates tested (n)
Organic	38	62	0	42
Small scale conventional	61	39	0	61
Large scale conventional	65	31	4	72
Total	57	41	2	175

Table 3. Metalaxyl resistance among isolates of *Phytophthora infestans* from different cropping systems in Estonia (2004-2005)

Cropping system	Metalaxyl resistance ^a			Total
	R (%)	I (%)	S (%)	
Organic	14	52	33	21
Small scale conventional	26	42	32	19
Large scale conventional	66	26	9	70
Total	49	34	17	110

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

Table 4. Frequencies of specific compatibility (virulence) to potato R-genes in isolates of *Phytophthora infestans* from different cropping systems in Estonia (2004–2005)

Crop system	Virulence to resistance gene											Mean number of virulences/isolate	Number of tested isolates
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11		
Organic	100	65	100	100	0	63	100	0	0	100	100	7.2	54
Small scale conventional	100	10	100	100	0	9	100	4	0	100	100	6.2	68
Large scale conventional	82	36	92	84	26	22	92	23	5	88	89	6.4	74
Total	95	38	98	95	5	31	98	4	1	97	97	6.6	196

Table 5. Race frequencies among isolates of *Phytophthora infestans* from different crop productions in Estonia (2004-2005)

Crop system	Races	Number of virulence factors	Number of isolates
Organic	1.2.3.4.6.7.10.11	8	34
	1.2.3.4.7.10.11	7	1
	1.3.4.7.10.11	6	19
Small scale conventional	1.2.3.4.6.7.10.11	8	6
	1.3.4.7.8.10.11	7	3
	1.2.3.4.7.10.11	7	1
	1.3.4.7.10.11	6	58
Large scale conventional	1.2.3.4.5.6.7.8.9.10.11	11	3
	1.2.3.4.5.6.7.9.10.11	10	1
	1.2.3.4.5.6.7.8.10.11	10	1
	1.3.4.5.6.7.8.10.11	9	1
	1.2.3.4.6.7.8.10.11	9	1
	1.2.3.4.5.7.8.10.11	9	2
	1.2.3.4.5.6.7.10.11	9	1
	1.3.4.5.7.8.10.11	8	1
	1.3.4.5.6.7.10.11	8	1
	1.2.3.4.7.8.10.11	8	3
	1.2.3.4.6.7.10.11	8	1
	1.2.3.4.5.7.10.11	8	1
	1.2.3.4.5.7.10.11	8	3
	1.3.4.7.8.10.11	7	5
	1.3.4.6.7.10.11	7	2
	1.3.4.6.7.10.11	7	1
	1.3.4.5.7.10.11	7	2
	1.2.3.6.7.10.11	7	1
	1.2.3.4.7.10.11	7	1
	1.2.3.4.7.10.11	7	3
	1.3.4.7.10.11	6	14
	1.3.4.7.10.11	6	5
	2.4.7.10.11	5	1
	1.3.7.10.11	5	1
	1.3.4.7.11	5	1
	1.3.4.10.11	5	1
	3.4.7.10	4	1
	3.4.5.7	4	1
	2.3.4.7	4	1
	1.3.7.11	4	1

Table 5. (*Continued*)

Crop system	Races	Number of virulence factors	Number of isolates
	7.10.11	3	1
	3.7.8	3	1
	3.7.10	3	1
	3.10.11	3	1
	1.7.11	3	1
	1.3.7	3	1
	6.10	2	1
	3.11	2	1
	3.10	2	1
	11	1	1
Total number of isolates			196
Total number of races			38

Table 6. Number and percentages of mitochondrial DNA haplotypes among isolates of *Phytophthora infestans* from different cropping systems in Estonia (2004-2005)

Crop system	Number (and percentage) of isolates				Total
	Ia	IIa	Ib	IIb	
Organic	24 (100)	0 (0)	0 (0)	0 (0)	24
Small scale conventional	16 (67)	8 (33)	0 (0)	0 (0)	24
Large scale conventional	9 (50)	7(39)	0 (0)	2 (11)	18
Total	49 (74)	15 (23)	0 (0)	2 (3)	66

Table 7. Genotypes, phenotypes and sources of Estonian isolates of *Phytophthora infestans* (2004–2005)

Multilocus genotype	Mating type	Haplotype	Race	Metalaxyl resistance ^a	Number of isolates	Year found	Crop system ^b
1	A1	Ia	1.2.3.4.6.7.10.11	R, I, S	10	2004	O, S, L
2	A1	Ia	1.3.4.7.10.11	I, R, S	8	2004	S, O
3	A1	Ia	1.2.3.4.7.10.11	I	2	2004, 2005	L, O
4	A1	Ia	1.2.3.4.5.6.7.8.10.11	R	1	2005	L
5	A1	Ia	1.2.3.4.5.6.7.10.11	R	1	2004	L
6	A1	IIa	1.3.4.7.10.11	I, R	4	2004, 2005	L, S
7	A1	IIa	1.3.4.5.6.7.8.10.11	I	1	2004	L
8	A1	IIa	1.3.4.5.7.10.11	I	1	2005	L
9	A1	IIIb	1.3.4.7.10.11	R	1	2004	L
10	A1	IIIb	1.2.3.4.7.8.10.11	S	1	2005	L
11	A2	IIa	1.3.4.7.10.11	R	3	2004	O, L
12	A2	Ia	1.2.3.4.7.10.11	R	1	2005	L
13	A2	Ia	1.3.4.6.7.10.11	I	1	2004	L
14	A2	Ia	1.3.7	S	1	2004	L
15	A2	Ia	1.3.4.7.10.11	0	7	2004, 2005	S, O, L
16	A2	Ia	1.2.3.4.5.6.7.8.9.10.11	R	1	2005	L
17	A2	IIa	1.2.3.4.5.7.10.11	R	1	2005	L
18	A2	IIa	1.2.3.4.5.7.8.10.11	R	1	2005	L

^a S, sensitive; I, intermediate; R, resistant.

^b L, large scale conventional fields; O, organic fields; S, small scale conventional fields.



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**THE QUESTION OF METALAXYL RESISTANCE ON
LATE BLIGHT FUNGUS IN ESTONIA.**

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The question of metalaxyl resistance on late blight fungus in Estonia

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Abstract. Metalaxyl containing fungicides are commonly used by Estonian potato growers because of their efficiency in control of potato late blight. Metalaxyl-resistant isolates have become an important part of the Estonian population of *Phytophthora infestans*. The aim of the current study was to measure the frequency of metalaxyl resistance among the Estonian population of *Phytophthora infestans* and to test the efficacy of metalaxyl-based fungicides in controlling potato late blight. 138 isolates of *Phytophthora infestans* were collected from conventional, experimental and small scale growers' fields in different parts of Estonia in 2003-2005 and were assessed for resistance to metalaxyl. Metalaxyl-resistant, intermediate and sensitive isolates were recorded at frequencies of 37.1, 45.4 and 15.1%, respectively. There were differences in frequency of metalaxyl resistance between different locations, years and different types of potato cultivations. Metalaxyl-resistant isolates were predominant (66.7%) on conventional potato fields in 2004 and 2005. Metalaxyl-resistant strains were not detected among isolates collected from the field where fungicide containing metalaxyl was not used. The increased frequency of metalaxyl-resistant isolates was detected in 2003-2005. Trials of late blight control with fungicides containing metalaxyl were performed in the same period. Metalaxyl showed lowered efficacy only in conditions of extreme late blight pressure in 2004. Therefore metalaxyl could be effectively used for control of potato late blight a maximum of two times during the growing period.

Key words: metalaxyl resistance, *Phytophthora infestans*, potato late blight, late blight control

INTRODUCTION

Phenylamide fungicides are a class of systemic compounds including metalaxyl (Ridomil, oxadixyl, benalaxyl and ofurace) that show excellent protective, curative and eradivative antifungal activity and exclusively control diseases caused by Peronosporales (Schwinn & Staub, 1987). An increase of metalaxyl-resistant isolates of *Phytophthora infestans* was observed between 1981 and 1984, the period during which phenylamide fungicides were suspended in Ireland and The Netherlands. The proportion of metalaxyl-resistant isolates fluctuates from year to year and within seasons (Gisi & Cohen, 1996). In single years the values varied significantly (between 0-100%), with maximum levels at 1987–90 in most countries. In 1990s, resistance levels remained more or less stable in all European countries (Gisi & Cohen, 1996).

Fungicides containing Metalaxyl are commonly used by Estonian potato growers because of their efficiency in the control of potato late blight. Metalaxyl-resistant isolates have become an important part of the Estonian population of *P. infestans*.

The aim of the current study was to measure the frequency of metalaxyl resistance among the Estonian population of *P. infestans* and to test the efficacy of metalaxyl-based fungicides in controlling potato late blight.

MATERIALS AND METHODS

Collection and culture of isolates

Potato leaves naturally infected by *P. infestans* were collected during 2003–2005 from different parts of Estonia. Samples originated from conventional fields (Ingliste), small-scale farm fields (different locations) and experimental plots of Jõgeva Plant Breeding Institute. Blighted leaves were collected starting from the emergence of disease until the end of growing season. Isolation was carried out from one typical, single lesion per sample. Isolations were attempted by transferring a fragment of infected plant tissue at first to potato slices or tubers of susceptible cultivars (Bintje or Berber) without known R-genes. Rye agar was used for long term preservation of isolates of *P. infestans*.

Response to metalaxyl

In total, 138 samples were analysed for resistance to metalaxyl. Leaves of greenhouse grown 4-5 week-old plants of susceptible cultivars Bintje or Berber were used. Leaflets were floated in distilled water or in solutions of technical metalaxyl of 10,0 and 100,0 mg L⁻¹ concentrations. Twenty microlitres of sporangial suspension was placed in the centre of each leaflet. Inoculated leaflets were incubated at 15°C for 7 days. Each isolate was tested in 3 replications. After incubation, the leaves were observed using a stereomicroscope to estimate fungal growth and sporulation. The isolates were rated as resistant if they sporulated on leaflets in 100 mg L⁻¹ metalaxyl. Those sporulating in a metalaxyl concentration of 10 mg L⁻¹ were rated intermediate, and those sporulating only in water (?) metalaxyl were rated sensitive.

Trials of late blight control

Field experiments were arranged at the Jõgeva Plant Breeding Institute in 2003–2005. Two potato varieties differing in resistance to late blight were used: moderately resistant Anti in 2003 and 2004 and susceptible Asterix in 2005. In all three years two different treatment regimes were used: untreated, where fungicides were not applied, and routine treatment where the first two sprayings used protectant fungicide Ridomil Gold MZ 68 WG (metalaxyl-M 40, mancozeb 640) 2.5 kg ha⁻¹ at 10–12 day intervals starting from row closing, and the next treatments with eradicator fungicide Shirlan (fluazinam 500) 0.4 l ha⁻¹ at 10 day intervals. Trials were laid out according to randomized block design in three replications. The plot size was four rows (7,5*0,7 m). To avoid possible neighbouring effects in border rows, late blight was estimated and the crop was harvested only from the two middle rows. Late blight infection was assessed twice a week according to 0–100% scale.

Statistics

The statistical analysis of frequencies of *P. infestans* in different categories was performed using MS Excel. The results of the analyses are expressed as probability

values (p) related to chi-square values. Late blight infection was expressed as an average percentage of infected leaf area.

RESULTS AND DISCUSSION

Metalaxyl-resistant, intermediate and sensitive isolates were recorded as 37.1%, 45.4% and 15.1% respectively. Statistically significant differences ($P < 0.001$) in the frequency of resistance to metalaxyl were observed between locations (Fig. 1), potato growing field types and years. The proportion of metalaxyl-resistant isolates from different locations ranged from 0 to 67% (Fig. 1). Metalaxyl-resistant isolates were predominant among isolates collected from the experimental field in 2004 (66.7%) and where metalaxyl was used on conventional fields in 2004 and 2005 (60%). In 2004 no metalaxyl-resistant strains were detected among isolates collected from the field (Naha) where fungicide containing metalaxyl was not used. As might be expected, insensitivity was more often found in conventional fields, where metalaxyl was commonly applied, than in small-scale growers' fields where fungicides were rarely used. The increase of frequency of metalaxyl-resistant isolates was detected from 2003-2005. Metalaxyl-resistant isolates were less frequent among isolates collected in 2003 than in 2004 and 2005. Even though the proportion of metalaxyl-resistant isolates fluctuates from year to year, there is strong evidence- that the quantity of metalaxyl-resistant isolates has increased compared with previous results (Runno et al., 2005). The reason for the increase could be the more consistent use of fungicides containing metalaxyl. As long as metalaxyl is used according to the recommendations in Estonia it is unlikely that metalaxyl-resistant isolates will completely displace sensitive isolates.

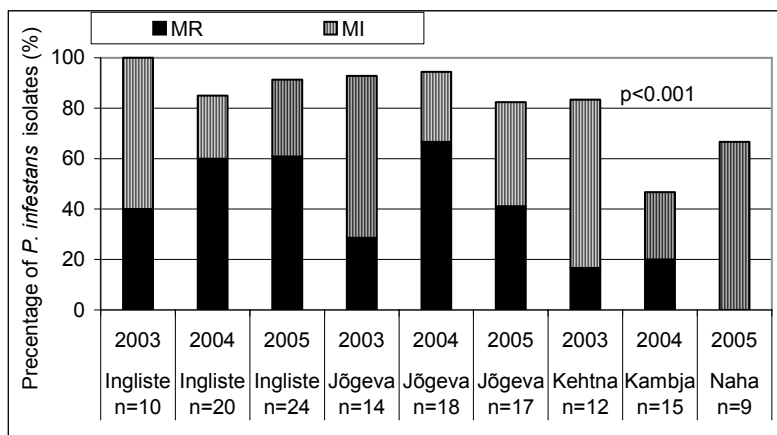


Fig. 1. Metalaxyl resistance^a among isolates of *Phytophthora infestans* from different locations in Estonia during 2003–2005.

^aMR, metalaxyl resistant; MI, intermediate metalaxyl sensitive.

Several reports suggest that resistance is unstable in the population and is selected anew each year, increasing steadily during the season and declining over winter (Gisi & Cohen, 1996).

Trials of late blight control with fungicide containing metalaxyl Ridomil Gold MZ 68 WG were performed in the same period. Metalaxyl-containing fungicide was applied 2 times in all three years. The foliage of untreated plots was completely destroyed by the end of the growing season in all trial years. Metalaxyl provided high protection in 2003 and 2005, but showed lowered efficacy only in conditions of extreme late blight pressure in 2004 when the foliage was infected while metalaxyl was in use (Table 2). The trial results show that in spite of the occurrence of resistant strains the use of fungicides consisting of metalaxyl is still effective. Therefore metalaxyl could be used effectively for control of potato late blight a maximum of two times at the beginning of the fungicide treatments. By following the application rules for metalaxyl fungicides it is possible to restrict the development of metalaxyl-resistant strains and thus avoid the reduction of efficacy of the fungicide.

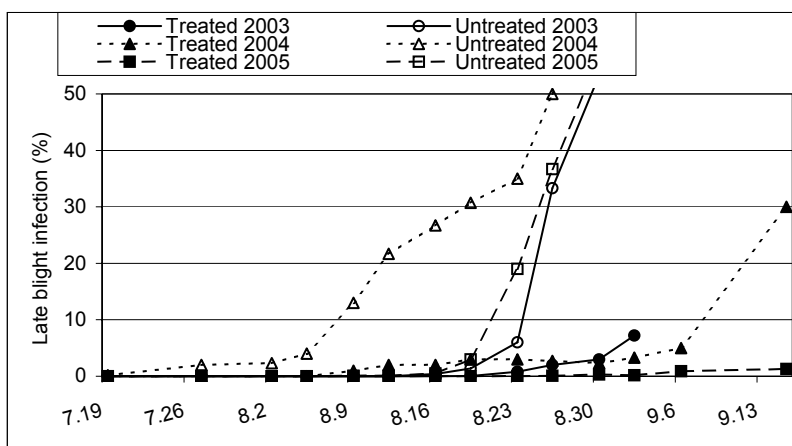


Fig. 2. Potato late blight infection assessments during 2003–2005.

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REFERENCES

- Gisi, U. & Cohen, Y. 1996. Resistance to phenylamide fungicides: a case study with *Phytophthora infestans* involving mating type and race structure. *Annual Review of Phytopathology* **34**, 549–72.
- Schwinn, F.J. & Staub, T. 1987. Phenylamides and other fungicides against Oomycetes. In Lyr, H. (ed.): *Modern Selective Fungicides*. Jena: VEB Gustav Fischer, pp. 259–73.
- Runno, E., & Koppel, M. 2005. *Phytophthora infestans* Eesti isolaatide metalaksüülitundlikkus ja paarumistüübid. *Agronomia* 2005. *Teadustööde kogumik* **220**, 168–170 (in Estonian).

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Education:

2002-2010 Estonian University of Life Sciences, doctoral studies in Plant Pathology
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Foreign languages:

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2009- Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, specialist
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- 2001-2004 Project ETF4737 „Occurrence of avirulence genes, fungicide resistance and mating types in Estonian population of *Phytophthora infestans*”. Principal investigator
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Other projects:

- 1998-2003 Development of environmentally friendly plant production technologies I”. Doctoral student
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2005 11.IV-16.VI	Characterization of genetic variation of <i>Phytophthora infestans</i> in Estonian population with molecular DNA markers. Agrifood Research Finland (MTT)
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2004 22-24.III	Course „Working with radioactive material in the laboratory”. Cornell University, USA
2003 28.II-30.IV	TOEFL Preparation course. Tartu University, Estonia
2001 13-15.VI	Good Experimental Practice Course; Tuhala, Estonia
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Täiendharidus:

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- 2005 11.IV-16.VI *Phytophthora infestans* geneetilise varieerumise iseloomustamine Eesti populatsioonis molekulaar DNA markeritega. Soome põllumajandus uuringute instituudis (MTT)
- 2004 22.II-8.VII Kristjan Jaak stipendiumi finantseerimisel Cornelly Ülikoolis (USA) prof W.E. Fry laboris doktoritöök vajalike *Phytophthora infestans*'i genotüüpiliste karakteristikute iseloomustamine ja selleks vajalike meetodikate omandamine ning teoreetilise baasi omandamine, Ithaca, USA
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LIST OF PUBLICATIONS

1.1. Publications indexed in the ISI Web of Science database:

- Runno-Paurson, E., Fry, W.E., Myers, K.L., Koppel, M., Mänd, M.** 2009. Characterization of *Phytophthora infestans* isolates collected from potato in Estonia during 2002–2003. *European Journal of Plant Pathology*, **124**, 565-575.
- Runno-Paurson, E., Fry, W.E., Remmel, T., Mänd, M., Myers, K.L.** 2010. Phenotypic and genotypic characterisation of Estonian isolates of *Phytophthora infestans* in 2004-2007. *Journal of Plant Pathology*, **91** (in print).
- Runno-Paurson, E., Remmel, T., Mänd, M., Ojarand, A.** 2010. Do crop management practices influence the structure of *Phytophthora infestans* population? *European Journal of Plant Pathology* (in review).

1.2. Papers published in other peer-reviewed international journals with a registered code:

- Runno-Paurson, E., Remmel, T., Koppel, M., Tähtjärv, T.** 2010. Occurrence and distribution mating types A1 and A2 of *Phytophthora infestans* in eastern Estonia. *Agronomy Research* (accepted for publication).
- Runno, E., Koppel, M.** 2006. The question of metalaxyl resistance on late blight fungus in Estonia. *Agronomy Research*, **4**, 341-344.

1.3. Papers in Estonian and in other peer-reviewed research journals with a local editorial board:

- Runno, E., Koppel, M.** 2002. NEGFYRY - kartuli lehemädaniku tõrjeprogramm. *Agraarteadus* 2(XIII), 96-100.

3.2. Articles/chapters in books published by the publishers not listed in Annex:

Koppel, M., **Runno-Paurson, E.** 2007. A simple DSS for control of potato late blight. H.T.A.M. Schepers Ed). Proceeding of the tenth workshop of an European network for development of an integrated control strategy of potato late blight (251-254). Wageningen: Applied Plant Research – Wageningen University and Research centre.

Runno, E., Koppel, M. 2006. An overview of the situation of the Estonian population of *Phytophthora infestans*. In Schepers, H.T.A.M. (Ed) Proceedings of the Ninth Workshop of an European network for development of an intergrated control strategy of potato late blight (157-164). Lelystad: Applied Plant Research.

3.3. Specific research publications/dictionaries, lexicons, sets of maps, (field) guides, text-critical publications):

Soobik, P., Koppel, M., **Runno, E.** 2006. Kartuli haigused ja kahjurid ning nende tõrje. Jõgeva: Jõgeva Sordiaretuse Instituut.

3.4. Papers published in the proceedings of international conferences:

Koppel, M., **Runno, E.**, Sooväli, P., Lauringson, E., Talgre, L., Nurmekiivi, H. 2003. Control of spring wheat diseases in meteorologically different conditions. Proceeding of the Crop Protection Conference for the Baltic Sea region. Regional collaboration and exchange of results regarding Crop protection and Pesticides. ICCN 1397-9884 (142-148). Danish Institute of Agricultural Sciences.

Runno, E., Koppel, M. 2002. Validation of Potato Late Blight control system NEGFY in Estonian conditions. Proceedings of International scientific conference of Research for Rural Development 2002, Jelgava, Latvia 61-64.

Koppel, M., **Runno, E.**, Talvoja, P. 2002. Characterization of *Phytophthora infestans* isolates collected from Estonia in 2001. Proceedings of the Scientific International Conference of Plant Protection in the Baltic Region in the Context of Intergation to EU, Kaunas, Lithuania, 58-60.

3.5. Articles/presentations published in local conference proceedings:

- Runno-Paurson, E., Koppel, M.** 2009. Muutunud lehemädaniku tekitaja. *Agronoomia* 2009, 186-191.
- Sooväli, P., Koppel, M., **Runno-Paurson, E.** 2008. Fungitsiidide efektiivsus talinisu ja odra haiguste tõrjel. Põllukultuuride uuemad sordid, nende omadused ja kasvatamise omapära (37-44). Jõgeva: OÜ Vali Press.
- Sooväli, P., **Runno-Paurson, E., Koppel, M.** 2007. Vähendatud fungitsiidinormide kasutamine teraviljahaiguste tõrjel. Millest sõltub teravilja saagikus (14-25). Jõgeva: OÜ Vali Press.
- Koppel, M., **Runno-Paurson, E.** 2006. Kartulisortide lehemädaniku-kindluse arvestamine keemilise tõrje ajastamisel. *Agronoomia* 2006, 124-129.
- Runno, E., Koppel, M., Talvoja, P.** 2005. *Phytophthora infestans* Eesti isolaatide metalaksüülitundlikkus ja paarumistüübid. *EPMÜ Teadustööde kogumik*. *Agronoomia* 2005, 168-170.
- Runno, E., Koppel, M.** 2005. Kartuli-lehemädaniku tõrje programm Neg-Fry. Sordiaretus ja seemnekasvatus. Teaduslikud tööd IX, 245-250.
- Koppel, M., **Runno, E.** 2005. Avirulentusgeenide, paarumistüüpide ja fungitsiidiresistentsuse esinemine kartuli-lehemädaniku tekitaja *Phytophthora infestans* populatsioonis. Sordiaretus ja seemnekasvatus. Teaduslikud tööd IX, 239-244.
- Runno, E., Koppel, M.** 2004. *Phytophthora infestans* patotüübid Eestis. *EPMÜ Teadustööde kogumik*. *Agronoomia* 219, 172-174.
- Runno, E., Koppel, M.** 2001. Kartuli-lehemädaniku tõrje programmi NEGFY kasutamine Eesti tingimustes. *EPMÜ Teadustööde kogumik* 213 (141-146).

6.3. Popular Science articles:

- Runno-Paurson, E.** 2010. Lehemädanik ründab järeljätmatult! Maa-kodu (Suve-eri,) 112-114.
- Runno-Paurson, E., Sildnik, K.** 2009. Kartulikasvataja põline nuhtlus on muutunud. Maalehe nõuandelisa Targu Talita.

- Runno-Paurson, E.** 2009. Kartulivähk rikub põllu pikaks ajaks. Maalehe nõuandelisa Targu Talita.
- Koppel, M., **Runno, E.** 2005. Ühisrinne kartulilehemädaniku vastu. Maamajandus 11(28-30).
- Runno, E.,** Narits, L. 2004. Eestis enam levinud rapsi- ja rüpsihaigused ning nende tõrje agrotehnilised võimalused. Eestimaa Talupidajate Keskliidu info- ja nõuandeleht Eesti Talu. Nr. 2, lk. 5-6.
- Sooväli, P., **Runno, E.** 2004. Iga plekk ei ole taimehaigus. Maamajandus 7(27-28).
- Koppel, M., **Runno, E.,** Nurmekivi, H. 2001. Kartuli-lehemädaniku tõrje programm NegFry. Maamajandus 6 (27-29).
- Runno, E.,** Sõmermaa, A.-L. 2000. Lutserni seenhaigused ja nende tõrje. Põllumajandus 6 (9-10).