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# The structure of *Phytophthora infestans* populations from organic and conventional crops

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**Abstract** The characteristics of populations of *Phytophthora infestans* from organic farms, small conventional farms and large conventional farms were determined from isolates collected in northern Estonia in 2004 and 2005. For the population as a whole 41% were A2; all virulence factors to the 11 R genes from *Solanum demissum* were found; and more than 70% had high or intermediate resistance to metalaxyl. Isolates from organic farms tended to have more complex pathotypes than isolates from either large or small conventional farms, but there was a higher proportion of metalaxyl resistant isolates from large conventional farms than from small conventional farms or from organic farms.

**Keywords** Crop systems · Mating type · Metalaxyl resistance · Virulence · Mitochondrial DNA haplotype · *Phytophtora infestans*

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## Introduction

Potato late blight, caused by the oomycete *Phytophthora infestans*, is one of the most devastating diseases of potato worldwide. It 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 destroying foliage and decreasing tuber growth, but also causes rotting of the tubers before and during storage (Smart and Fry 2001), thereby causing considerable further yield losses. In Estonia, it is not possible to achieve high yield with good quality in conventional potato production without using fungicides to control the late blight pathogen (Koppel 1997). In organic fields, where mostly varieties with high resistance are used, yield loss may reach 50% (Runno-Paurson *et al*, unpublished data). 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, these 'old' population genotypes have not been detected. The 'new' genotypes comprise isolates of both mating types (Spielman et al. 1991; Day and Shattock 1997; Lebreton and Andrivon 1998); their coexistence allows the pathogen to reproduce sexually and to form oospores. Oospores can withstand unfavourable conditions and survive in the soil, thus affecting the epidemiology of the disease (Mayton et al. 2000). The new population also contains both Ia and IIa mtDNA haplotypes (Day and Shattock 1997; Lebreton and Andrivon 1998). 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 et al. 1998; Schöber-Butin 1999; Andersson et al. 1998; Brurberg et al. 1999; Lehtinen et al. 2007; Lehtinen et al. 2008; Avendaño Córcoles 2007; Śliwka et al. 2006). The proportion of A2 mating type isolates collected from commercial potato fields has remained low in France, Germany, Belgium and Switzerland (Gisi and Cohen 1996; Bakonyi et al. 2002a), whereas in the Netherlands, the Nordic countries and the UK it has exceeded 50% (Hermansen et al. 2000; Turkensteen et al. 2000; Lehtinen et al. 2007; Lehtinen et al. 2008, Lees et al. 2009).

In Estonia, the A2 mating type was first found in 1987. Data from 2002–2003 indicated the presence of both mating types at most study 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 (Hannukkala and Lehtinen 2005).

The number of organic farms in Estonia has increased since the early 1990s, notably since 2002. About 10 percent of all cultivated land is used for potato production. However, organic farms in Estonia are varied; for example, many of them do not rotate crops and the seed potatoes they use are often not certified. More importantly, with the prohibition of fungicide use, organic farms have a higher risk of late blight epidemics and consequent yield loss than conventional fields.

The main objective of this study was to compare the population structure of *P. infestans* in organic and conventional productions in Estonia. It was postulated that *P. infestans* populations in organic production may differ in their resistance to fungicides or the diversity of certain phenotypic or genotypic traits (mating types, specific virulence, resistance to metalaxyl and mtDNA haplotypes) from those in conventional production. Higher diversity is likely to pose a higher risk of yield loss; for example, when two mating types co-occur and produce oospores, there is a higher risk of long-term survival of the pathogen. A higher diversity in virulence or fungicide resistance related traits can lead to a more effective selection of these traits. 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 production) in northern Estonia (Table 1). The small and large scale conventional farms sampled differed in their use of agro-technical methods. In the small scale conventional farms, farmers used seed potatoes of uncertain quality and did not practise good crop rotation. Fungicides were applied only once per growing season. In the large scale conventional farms, farmers used high-quality certified potato seed, adhered to the recommended crop rotation, and made at least 6–7 treatments against potato late blight per season. Copper based fungicides are not used in Estonian organic production.

Nine to twenty-three leaflets, each with a single lesion (one per plant) were collected in organic and small scale farms twice in each year: at the beginning of the outbreak and at the end of the growing season (an approximately equal number of isolates was taken early and late in the season). In the early stages of the outbreak, approximately 20–25% of leaf area of the

**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 |

infected plants and less than 10% of plants were infected with late blight. In the later stages, about 20–40% of the leaf area and more than 50% of the plants were infected. On the large scale farms, samples were collected at the beginning of the 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 and Jinks 1968). The pure cultures were preserved at 5°C and transferred to rye agar after every 2 months. All phenotypic tests were carried out in October–November of the year of isolation. Mitochondrial DNA haplotype analyses were conducted in November–December 2005.

We used the data of field tests for foliage blight resistance (scores 1–9) from the EUCABLIGHT database (www.eucablight.org) to evaluate the late blight resistance of potato varieties grown in our study fields.

## Phenotypic analyses

Mating types were determined by the method described in Runno-Paurson et al. (2009). Observed oospore formation in single isolate pure cultures was interpreted as the occurrence of self-fertility of the isolates. The tester isolates were the same as those described in Lehtinen et al. (2007). The resistance to metalaxyl of all 70 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 196 isolates was determined using Black's differential set of potato genotypes containing resistance genes R1–R11 (Malcolmson and Black 1966) (provided by Scottish Agricultural Science Agency). Laboratory procedures were performed as described in Runno-Paurson et al. (2009).

## Neutral marker assessment

At least four isolates were selected from each field (66 in total) for determination of the mitochondrial DNA (mtDNA) haplotype. 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) method of Griffith and Shaw (1998). The mtDNA haplotype detection was conducted at 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 with that of standard solutions on agar plate containing ethidium bromide under UV light. A DNA concentration of 1 ng/μl or more in the PCR mix was required to get clear PCR product bands in the following electrophoresis. The DNA polymerase DyNAzyme II (Finnzymes) was used with a concentration of 0.02 U/μl in the PCR mix. The primers used for amplification of mt DNA regions P2 and P4 were F2 (5′-TTCCCTTTGTCCTCTACCGAT-3′)+R2 (5′-TTACGGCGGTTTAGCACATACA-3′) and F4 (5′-TGGTCATCCAGAGGTTTATGTT-3′)+R4 (5′ CCGATACCGATACCAGCACCAA-3′), 0.2 p mol/μl. The PCR program used for amplification of P2

was 1x 90°C for 5 min, 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 30 s and 4°C for ever). For P4, an annealing temperature of 55°C was used. Digestions with MspI (P2) and EcoRI (P4) were performed at 37°C overnight. The digested DNA samples were loaded into 1% agarose gel containing ethidium bromide. The restriction patterns were visualized using an UV transilluminator.

### Data analysis

Statistical analyses were performed with the 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. Average susceptibility (scores 1–6, variety-specific scores were obtained from the Potato Late Blight Network For Europe website calculated from the results of foliage blight field tests) of potato plants grown in the different cropping systems to late blight was compared using a logistic model with an ordinal multinomial response variable. Race diversity was calculated with the normalized Shannon diversity index (Sheldon 1969).

## Results

### Mating type determination

Among the 175 tested isolates, 57% were A1 mating type, 41% were A2 mating type and 2% were self-fertile. Both A1 and A2 mating types were detected from 11 of the 12 fields. The proportion of the A2 mating type in the isolates sampled in 2004 was lower than those sampled in 2005 (28% resp. 54%; $\chi^2=11.87$, 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 2 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 the large scale conventional fields, 66% of the tested isolates were resistant to metalaxyl, while in the small scale farm fields 26% and in the organic fields only 14% of the isolates were resistant (Table 3). There were no differences between 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, $p=0.02$).

**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] | | | |
|---|---|---|---|---|
| | R (%) | I (%) | S (%) | Total |
| 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

## 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). A difference in the prevalence of virulence factors 2, 5, 8, and 9 was observed between the two sampling years (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 remained significant. The three rarest virulence factors in Estonia, R5, R8 and R9, only appeared in the large scale conventional fields, while virulence factors R2 and R6 with relatively low frequencies were more prevalent in the organic fields than in the other cropping systems. 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 highest in the organic farms (7.3). Complex races predominated in the organic fields, but were less common in the small and the 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 the large scale conventional fields, but much lower in the small scale (0.13) and the organic fields (0.18).

Potato plants grown in large scale farms were on average less resistant to late blight than those grown in small scale and organic farms (Wald.stat=80.18, d.f.=2, $p$<0.01).

## Mitochondrial DNA haplotype

Three mitochondrial haplotypes (Ia, IIa and IIb) were detected among the 66 isolates tested. Two isolates of haplotype IIb were found from the large scale

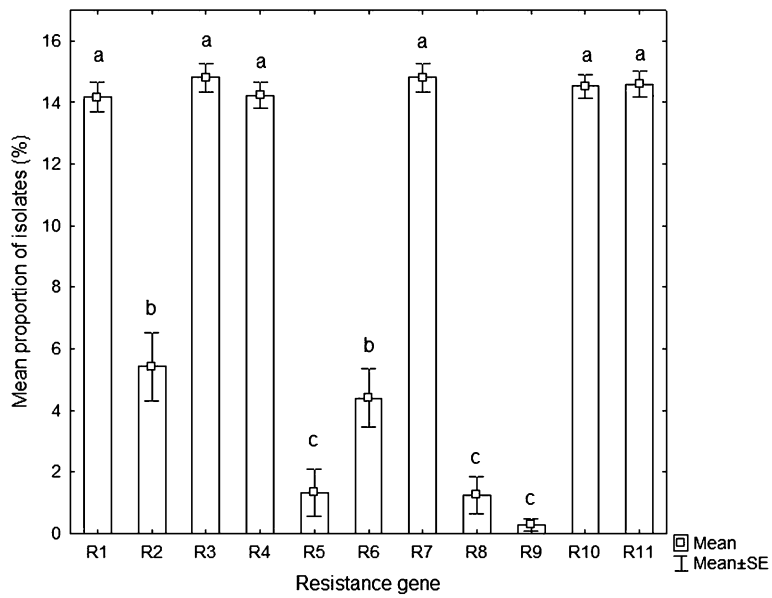


**Fig. 1** Frequency (percentage) of virulence to potato R-genes among isolates of *Phytophthora infestans* collected from different cropping systems in Estonia (2004–2005)

**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 |

conventional fields. The majority of isolates were haplotype IIa (74%) and the 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 the large scale conventional fields and lowest in the 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).

## Discussion

The results of this study suggest that there may be differences between potato cropping systems in various aspects of the population structure of *P. infestans* present in the fields. It is probable that different management practices, mainly fungicide use but also crop rotation and the source of potato seeds, are behind these differences. Dissimilarities were found in the prevalence of mating types, virulence genes, mtDNA haplotypes and resistance to metalaxyl. These results were also not always in accordance with those found in previous studies in this and 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; Runno-Paurson et al. 2010). Somewhat lower prevalence of the A2 mating type has been reported in Belgium (Bakonyi et al. 2002a), 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 (Avendaño 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 (Śliwka 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 at nearly all sites (92% of studied fields), with a single exception of an organic field in 2004. This percentage is based on just twelve fields, but it is supported by previous studies, conducted in 2002–2003 and 2004–2007, based on 32 and 28 fields, respectively (Runno-Paurson et al. 2009; Runno-Paurson et al. 2010), where the two mating types co-occurred in 88% of the fields. Similar frequencies of co-occurrence of the mating types have been reported from Germany (Bouws and Finckh 2007) where two mating types co-existed in 60–92% of the sites, and frequencies as high as 29–56% have been found in Nordic countries (Lehtinen et al. 2008). However, it is possible that the differences between studies arise from different numbers of isolates studied per field, rather than true differences in population composition, as the probability of detecting both mating types depends on sample size. This study did not support the previous findings that the co-occurrence of both mating types is more common

**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 |
| | 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 |

**Table 5** (continued)

| Crop system | Races | Number of virulence factors | Number of isolates |
|---|---|---|---|
| | 3.11 | 2 | 1 |
| | 3.10 | 2 | 1 |
| | 11 | 1 | 1 |
| Total number of isolates | | | 196 |
| Total number of races | | | 38 |

in organic fields, as has been reported from Finland (Lehtinen et al. 2007), southern Flevoland in the Netherlands (Zwankhuizen et al. 2000) and Scotland (Cooke et al. 2003). However, 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 the organic fields, 62% of isolates were A2 mating type whereas in the large scale conventional farm fields only 31% of isolates were A2 mating type. 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 in the organic fields than in the other cropping systems. Organic fields were also more severely infected than conventional crops, even though less susceptible potato varieties were used. The main reason for this is probably the lack of fungicide use in organic fields; however, an additional risk factor may be an increased oospore production, which reduces the effect of crop rotation if it is not performed sufficiently frequently (Lehtinen et al. 2007).

Further differences between cropping systems were evident in the resistance of isolates to metalaxyl fungicides. Metalaxyl resistant isolates were found four times more often in the large scale conventional fields than in the organic fields. This difference could be explained by the use of metalaxyl products in the large scale conventional fields, even though no significant differences were detected between the large scale conventional fields treated and not treated with metalaxyl (statistics not shown). Furthermore, it is possible that the overall prevalence of metalaxyl resistance has varied in recent years; in a previous study (Runno-Paurson et al. 2009), the average percentage of resistant isolates was 30%, but, in the present study was 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 intensive use of metalaxyl in Estonia against the heavy late blight pressure during those years may have contributed to this variation in resistance, although we cannot be certain that the difference is not coincidental.

The Estonian population of *P. infestans* is most similar in the frequency of virulence factors to those described recently in Nordic countries (Hermansen et al. 2000; Lehtinen et al. 2007, 2008), France and Switzerland (Lebreton and Andrivon 1998; Knapova and Gisi 2002; Pilet et al. 2005). The mean number of virulence factors found in Estonia (6.6) has remained at approximately the same level as in previous years (Runno-Paurson et al. 2009; Runno-Paurson et al. 2010); similar values were also found in Denmark (6.92) and Sweden (6.87) (Lehtinen et al. 2008) in 2003. Pathotype variability seems to have increased

**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 | | | | |
|---|---|---|---|---|---|
| | Ia | IIa | Ib | IIb | Total |
| 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 |

from the early 1990s in Norway and Finland from 5.3 and 5.8, respectively, (Hermansen et al. 2000) to 6.3 by 2002 (Lehtinen et al. 2007). A possible increase in the average number of virulence factors per isolate has also been noted in North-Western Russia since the late 1990s: from 6.3 to 7.7 in 2003 and 8.1 in 2007 (Vedenyapina et al. 2002; Zoteyeva and Patrikeeva 2008). However, the data are too few to rigorously confirm such an increase.

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). As a comparison, in a sample of 432 isolates collected in 2004–2007, pathogen diversity was still relatively low (0.54) (Runno-Paurson et al. 2010). Interestingly, even though lower values of diversity have been found in newer studies, the average virulence complexity was relatively high. The diversity index was much higher among isolates collected from large scale conventional fields. This result is particularly surprising because, unlike smaller farms, the large scale farms used certified potato seed tubers and practiced rotation. The reason for this may lie with the seed source used in those farms. Large scale farms grow potato varieties imported directly from western Europe, mostly from the Netherlands, where the local populations have highly complex virulence spectra (8–10 virulence factors per isolate) and the proportion of A2 is extremely high (Van Raaij et al. 2008). Large quantities of seed potato are also imported from Germany and Denmark. The mean number of virulences per tested isolate was found to be 6.9 in Denmark and 6.2 in Germany, and the frequency of A2 mating type was over 50% in Denmark and 13–46% in Germany, with both mating types co-existing in 76% of the fields, on average (Bouws and Finckh 2007; Lehtinen et al. 2008). It is therefore likely that the higher diversity of the *P. infestans* populations in large scale farms is caused by mixing local genotypes with strains imported from other, highly diverse populations.

Moreover, compared to small conventional and organic farms, the potato varieties used in large conventional farms were on average more susceptible to late blight, which may have contributed to the high pathogen diversity. A high variability of pathogens does not always pose a higher threat to the hosts, even though it can potentially promote the pathogen population to adapt more quickly with new host plant varieties, or to express virulence against a higher number of resistance genes. Nevertheless, a single pathotype can cause severe damage, as in the case of Great Britain where 80% of the population consists of only one aggressive genotype 13_A2, determined by SSRs and mating type (Lees et al. 2009). It can also be noted that, even though pathogen diversity tended to be higher in large conventional fields in our study, the plants were still more affected in organic fields.

Another dissimilarity found between cropping systems was the higher prevalence of the generally less common IIa haplotype in the large conventional fields compared to the other field types. This may also be explained by a larger number of imported pathogens in those fields. 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 (Lebreton and Andrivon 1998; Cooke 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 markers used were chosen to show mainly phenotypic variability, with genetic variation characterized by mtDNA haplotypes. The occurrence of A1 mating type isolates with three different mtDNA fingerprints clearly indicates that there is some genotypic diversity in the population. In a previous study (Runno-Paurson et al. 2010), the Shannon index of genotypic diversity, obtained by DNA fingerprinting with probe RG57, was particularly high in large conventional fields. In addition, the high numbers of rare genotypes detected every year indicate that oospores may act as an infection source in organic and conventional potato fields (Zwankhuizen et al. 2000). 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 pathogen diversity, such as genotypic diversity, race complexity and the diversity of mtDNA haplotypes appeared to be highest in the large 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 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. 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. 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. Importantly, the separate effects of crop rotation, chemical control, seed source and host resistance need to be addressed.

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