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The phenotypic characteristics of Estonian populations of *Phytophthora infestans* from organic and conventional potato crops

E. RUNNO-PAURSON[1,2]

[1]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences,
Kreutzwaldi 1, Tartu 51014, Estonia
[2]Department of Biochemistry and Plant Protection, Jõgeva Plant Breeding Institute,
Jõgeva alevik 48309, Estonia

SUMMARY

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 and metalaxyl resistance. 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. The results indicated several differences between cropping systems in the population structure of *P. infestans*. 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.

KEYWORDS

Phytophtora infestans, crop systems, mating type, metalaxyl resistance, virulence

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). 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organic fields, where mostly varieties with high resistance are used, yield loss may reach 50% (Runno-Paurson *et al.*, unpublished data).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.

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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 & 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 traits from those in conventional production. 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

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. The small and large scale conventional farms sampled differed in their use of agrotechnical 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 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 like described by Runno-Paurson *et al.,* 2009. Mating types were determined by the method described in Runno-Paurson *et al.,* 2009 and Runno-Paurson *et al.,* 2010. 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 & Black, 1966) (provided by Scottish Agricultural Science Agency). Laboratory procedures were performed as described in Runno-Paurson *et al.,* 2009.

RESULTS AND DISCUSSION

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%). There were further differences between cropping systems, the proportion of A2 being highest in organic fields and lowest in large scale conventional fields (Figure 1).

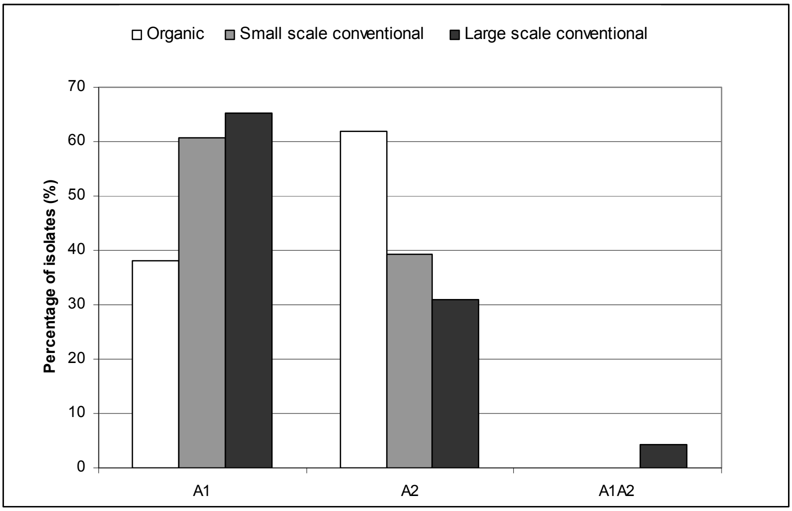

Figure 1. *Percentages of mating types among isolates of Phytophthora infestans from different cropping systems in Estonia (2004-2005).*

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). 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 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).

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.

Considerable differences between potato cropping systems were observed. In particular, in the large scale conventional fields, 66% of the tested isolates were resistant to metalxyl, while in the small scale farm fields 26% and in the organic fields only 14% of the isolates were resistant. There were no differences between years; 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% .

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).

Virulence

All known virulence factors (to overcome genes R1-R11) were found among tested 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. A difference in the prevalence of virulence factors 2, 5, 8, and 9 was observed between the two sampling years. 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 (Figure 2).

Thirty-eight races were detected. The two most common races made up 70% of the isolates tested. The overall virulence complexity (average number of R-genes overcome) was 6.7. Virulence complexity was highest in the organic farms. Complex races predominated in the organic fields, but were less common in the small and the large scale conventional fields.

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; Hermansen *et al.*, 2008), France and Switzerland (Leberton & Andrivon, 1998; Knapova & 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.

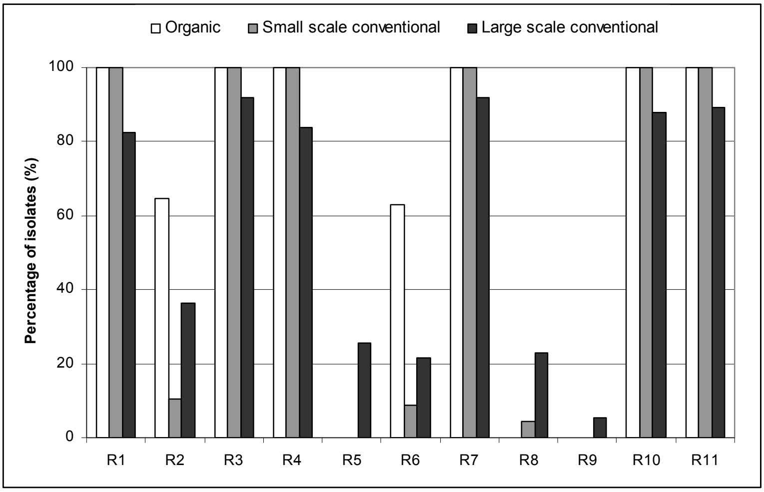

Figure 2. *Frequencies of specific compatibility (virulence) to potato R-genes in isolates of Phytophthora infestans among different crop productions in Estonia (2004-2005).*

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 & 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.

CONCLUSIONS

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 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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