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Chosen characteristics of Polish *Phytophthora infestans* isolates

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SUMMARY
Isolates of *P.infestans* collected in Poland from 2007 to 2009 were characterized by mating type, virulence, resistance to metalaxyl and mitochondrial DNA haplotype. In total 357 isolates were isolated, originating from different Polish regions. Majority of them were collected in 2009 when the late blight epidemic was very strong in Poland. Collected isolates were tested for mating type and of total 197 isolates 54 were of A1 mating type and 143 of A2 mating type. Two mitochondrial DNA haplotypes were detected in isolates - Ia and IIa, with Ia dominating – among 135 isolates tested 124 were of Ia mtDNA haplotype and 11 of IIa. In total 281 isolates were tested for metalaxyl resistance, and 211 were sensitive, 39 intermediate and 31 resistant. Virulence factors were frequent for genes *R1, R3, R4, R7, R10* and *R11*, moderately frequent for genes *R2*, *R6* and *R8,* and rare for *R5* and *R9*.

KEYWORDS
Phytophthora infestans, late blight, mating type, mitochondrial DNA haplotype, virulence, metalaxyl resistance

INTRODUCTION

Phytophthora infestans is the most destructive pathogen of potato and tomato worldwide, total losses in European Union are estimated to be around 1 billion Euro yearly – that include loss of crop, cost of fungicides (Haverkort, 2008). Chemical control of late blight is successful in high input potato production, but in Poland many small fields are left without any fungicide protection at all, and those can be totally destroyed by *P. infestans.* Before the 1980s worldwide population of late blight pathogen (with exception of those in central Mexico) consisted of only A1 mating type, which excluded sexual reproduction. Furthermore, population around the world was dominated by single clonal lineage – US-1. This situation was disrupted by migrations of A2 mating type and several clonal lineages from central Mexico, which occurred probably in the 1980s and early 1990s (Fry, 2008, Fry *et al.* 2009). Presence of both mating types made it possible for *P. infestans* to reproduce sexually and contributed greatly to genetic diversity of pathogen population worldwide and US-1 was replaced in many locations by new clonal lineages. Recently new clonal lineage called 13_A2 has increased its frequency in Europe, in years 2007-2009 it was found in many countries (United Kingdom, Netherlands, Germany, Switzerland, France, Poland), in United Kingdom it is dominating (www.eucablight.org, Cooke *et al.* 2010). In our institute we're monitoring late blight

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population in Poland, testing its characters – both phenotypic and genetic ones. Information about the pathogen such as resistance to common fungicides, mating type distribution and virulence factors are required to find most effective strategy against late blight.

MATERIALS AND METHODS

Isolation of *P.infestans* pure cultures

P. infestans was isolated from single lesions from potato leaflets using the procedure described by Śliwka *et al.* (2006).

Mating type determination

Mating type was determined by crossing tested isolate with A1 (MP 503) and A2 (US-8 isolate kindly supplied by W.Fry) isolates on ryeA agar medium with addition of β-sitosterol in concentration of 40 mg/l (Spielman *et al.*, 1990) and after incubation period of 10-14 days microscopic observation of oospore formation.

Virulence evaluation

Virulence was tested on 11 Black's differentials, each with single R gene (*R1-R11*), in detached leaflet assay (Zarzycka, 2001). Black's differential set was obtained from SASA, Edinburgh. Potato cultivars Sarpo Mira, Bzura and Biogold were used as additional differentials, together with wild potato species *S. ruiz-ceballosii* syn.*Solanum sparsipilum*(*rzc* 99-10/36) andapotato breeding line containing *Rpi-phu1* gene (04-IX-21).

Metalaxyl resistance

Resistance to metalaxyl was tested on agar plates with rye A medium, by measuring diameters of *P. infestans* cultures. Three variants of medium were made – without metalaxyl (control) and two with metalaxyl at final concentration of 5 and 100 mg/l (Metalaxyl-M, Syngenta Crop Protection). Isolates were classified as sensitive when diameters of culture on both 5 and 100 mg/l of metalaxyl were smaller than 40% of control. Intermediate isolates grew above 40% of control on 5 mg/l medium and below 40% of control on 100 mg/l medium. Resistant isolates achieved more than 40% of the control on both 5 and 100 mg/l medium (Bakonyi *et al.* 2002; Perez *et al.* 2001; Daggett *et al.* 1993). Standard isolates from each group of metalaxyl resistance were used along with tested isolates.

Mitochondrial haplotype

Selected isolates were grown of rye A liquid medium for 3-4 weeks, then the obtained mycelia were rinsed in sterile water, frozen and lyophilized. Using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) DNA was extracted, and then test for mitochondrial haplotype was done according to method described by Griffith and Shaw (1998).

RESULTS AND DISCUSSION

Mating type determination

197 *P. infestans* isolates from 2007 (36 isolates), 2008 (84 isolates) and 2009 (77 isolates) were tested for mating type. Relation of A1 to A2 in 2007 was 12 to 24 respectively, in 2008 it was 21 to 63 and in 2009 it was 23 to 67. In total 56 isolates represented A1 mating type and 154 represented A2 mating type.

Mitochondrial haplotype

Subset of 33 isolates from 2007, 50 isolates from 2008 and so far 52 isolates from 2009 were tested for mitochondrial haplotype. Among Polish *P. infestans* isolates two mitochondrial DNA haplotypes were detected – Ia and IIa. Results were as follows: among isolates from 2007 ratio of Ia haplotype to IIa was 28 to 5; among isolates from 2008 ratio was 49 to 1; among isolates from 2009 ratio was 47 to 5. In total 135 isolates were tested, 124 (92%) of them represented Ia haplotype and 11 (8%) represented IIa haplotype. Neither Ib nor IIb haplotypes were detected.

Virulence evaluation

All 11 virulence factors were observed among tested isolates (Figure 1). Virulence factors corresponding to genes *R1, R3, R4* and *R7* were found in above 95% of isolates, with exception of 2007 when percent of isolates virulent against *R1* was slightly below 80%. Virulence against *R10* and *R11* was also very common, ranging from 70% for *R10* in 2007 to over 90% for *R11* in 2008. Virulence factors against *R2*, *R6* and *R8* were found in between 20% and 50% of tested isolates. Virulence for *R5* and *R9* was rare, up to 10% of tested isolates with exception of *R9* in 2008 when it was slightly above 10% and *R5* in 2007 when it achieved just below 30% of tested isolates. Additional differentials used were cultivars Bzura, Sarpo Mira and Biogold, potato breeding line containing *Rpi-phu1* gene (marked as 04-IX-21) and one wild potato species *S. ruiz-ceballosii* syn. *S. sparsipilum*(marked as rzc 99-10/36). Number of isolates able to infect cultivar Bzura increased – in 2007 less than 30% of tested isolates were able to infect Bzura and in 2009 that ratio reached above 50%. In case of cultivar Sarpo Mira also increase of isolates able to infect was observed in 2009, from around 20% to around 35%. The biggest number of isolates infectious to cultivar Biogold was observed in 2008, when it reached just below 40%, in 2007 and 2009 that ratio was below 20%. Breeding line 04-IX-21 had very few infectious isolates, in every year number of virulent isolates was below 5%. In the case of wild potato species rzc 99-10/36 increase in number of infectious isolates was observed in 2009, but in was still below 10%.

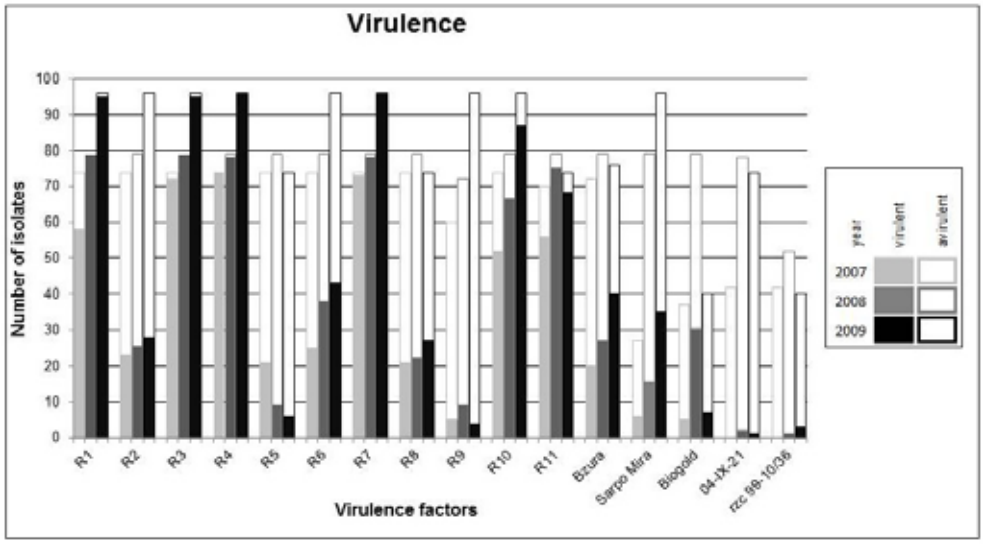

Figure 1. *Number of virulent and avirulent P. infestans isolates collected in 2007, 2008 and 2009 in Poland*

Metalaxyl resistance

In total 281 isolates were tested – 31 from 2007, 85 from 2008 and 165 from 2009. Results: in 2007: 5 resistant, 4 intermediate and 22 sensitive; in 2008: 6 resistant, 10 intermediate and 69 sensitive; in 2009: 20 resistant, 25 intermediate and 120 sensitive (Figure 2). In total 31 isolates were resistant, 39 intermediate and 211 sensitive, which in percentage is 11%, 14% and 75%, respectively.

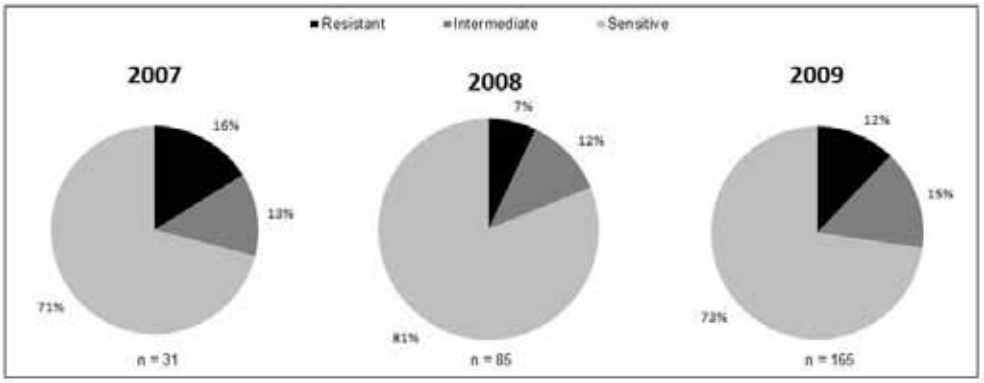

Figure 2.. *Resistance to metalaxyl of P. infestans isolates from 2007-2009*

DISCUSSION

Frequency of mating types of Polish *P. infestans* population has changed in last few years – in studies made in 2002-2004 A1 mating type was dominating with 61% against 39% of A2, higher frequency of A1 was also observed among isolates collected before 2002. Yet, from 2005 domination of A2 mating type has been observed – among isolates from 2005 and 2006 A2 appeared in 66% of tested isolates (Śliwka *et al*, 2006; Lebecka *et al*, 2007). And in years 2007-2009 this tendency continues with 73% of A2 mating type. Results from www.eucablight.org show that domination of A2 mating type occurred in most of countries for which data were published on this website. In Poland neighbouring countries distribution of mating types is variable – in Germany A2 dominated largely in 2007, in Slovakia data from 2003 shows about 60% of A2 mating type, but in 2004 only A1 mating type was detected. In Czech Republic results from 2003-2005 show distribution of mating types near to 1:1 (Mazakova *et al.,* 2006)

Virulence factors against *R1, R3, R4, R7, R10* and *R11* were found in most of tested isolates, which is very similar to results from 2005-2006 (Lebecka *et al*, 2007). Virulence factors against *R6* and *R8* also occurred with similar frequency as in previous studies, but against *R2* appearance was lower, in the case of *R5* and *R9* virulence was kept on low level. When isolates were tested against breeding line containing *Rpi-phu1* gene very small number appeared to be infective. This could happen because potatoes with this gene are rarely cultivated and therefore selection pressure on pathogen is relatively low. Another explanation could be that Avr factor corresponding to this gene is conservative and rarely undergoes changes, which would make resistance coming from *Rphi-phu1* gene durable and very useful for potato breeding. Increasing number of isolates able to infect cultivars Bzura and Sarpo Mira could be a problem for potato growers, especially that both those cultivars are supposed to be highly resistant to late blight (Haynes *et al.*, 1998; Hansen *et al.,* 2006). Infectivity of isolates against cultivar Biogold remains relatively low.

Results of virulence from Polish isolates are consistent with other European countries when comparing virulence factors against *R1, R3, R4, R7, R10* and *R11,* yet there are differences against rest of R genes from Black's differentials. In Belgium in years 2005-2008 virulence factors against

R2 and *R6* were found in above 50% of tested isolates and there were no isolates containing virulence factors against *R5, R8* and *R9.* In Denmark in data from 2003 virulence factors against *R6* were found in more than 50% of tested isolates, and no isolates containing virulence factors against *R9.* In Estonia according to data from 2004 to 2007 frequency of virulence factors are very similar to those in Poland, with exception of *R2* – in Estonia around 50% of isolates had virulence factors against this gene. In Finland virulence factors against *R2, R5, R6, R8* and *R9* are found very rarely, all below 10%. In France frequency of virulence is similar to those in Finland, but virulence against *R8* is higher – around 20%, and no isolates were tested against *R9,* therefore there is no information available. In Slovakia frequency of virulence against *R2* is above 50%, against *R8* around 60%, and against*R9* no virulent isolates were found (www.eucablight.org).

Resistance to metalaxyl was relatively low – 75% of isolates were sensitive, which is similar to results from 2005 and 2006 when 73,3% of isolates were sensitive, and to results from 2002 to 2004 when 82% of isolates were sensitive. Yet, lack of data concerning usage of fungicides on fields which were sources of isolates in years 2007 – 2009 prevents us from drawing conclusions. Low percentage of resistance to metalaxyl among Polish isolates was observed, compared to data from other European countries. For example in France resistant isolates dominated the population (www.eucablight.org). In Poland losses caused by late blight is estimated at 39,4%, mostly on fields with no chemical protection at all, which are still in majority in Poland (51,2-61%) (Kapsa, 2004) – therefore on those fields there is no pressure on pathogen to maintain resistance to fungicide – that could explain very low number of isolates resistant to metalaxyl in Polish population of *P. infestans*. Very similar frequency of resistance to metalaxyl was observed in Czech Republic, in years 2003-2005 81% of tested Czech isolates were sensitive to metalaxyl (Mazakova, unpublished data).
Polish population of late blight is heavily dominated by isolates with Ia mitochondrial haplotype with 92% of tested isolates representing this haplotype. Ia dominance also appeared in previous studies, when in group of 74 isolates from years 1997 to 2006 Ia haplotype was represented by 66 isolates (89%). Ratio of Ia haplotype dominance in Poland seem to be constant, and no Ib or IIb haplotypes were found. General tendency of Ia haplotype domination is common for most of Europe, with exception of Northern Ireland where IIa haplotype is more frequent (www.eucablight.org).

CONCLUSIONS

Polish population of *Phytophthora infestans* is complex and diverse. In most cases it's very similar to population in Europe – proportions of mating type, mitochondrial haplotype and most of virulence factors are very similar. Yet, the frequency of resistance to metalaxyl of Polish population differs from European populations.

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