VIRULENCE AND MATING TYPE OF *PHYTOPHTHORA INFESTANS* ISOLATES IN THE CZECH REPUBLIC*

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To evaluate the frequency and stability of the occurrence of *P. infestans* races and mating types in the Czech Republic, 338 monosporic isolates were collected from 31 sites in different potato-growing areas from 2012 to 2014 and in 2016. In total, 142 isolates were evaluated for virulence and race structure using the detached leaflet assay on Black's differential set, supplemented with cultivar Sarpo Mira and somatic hybrid REG 46F. With the exception of virulence for resistance genes *R9* and *Rpi-blb-1*, all virulence genes were detected among isolates, with a predominance of genes *R1*, *R3*, *R7*, *R10,* and *R11*. Most isolates were virulent to five or more *R*-genes, with a mean virulence complexity of 7.1. Among the 38 races detected, the most commonly occurring races were 1.2.3.4.6.7.10.11 and 1.2.3.4.7.10.11. Of the 338 isolates tested by the pairing test and the cleaved amplified polymorphism sequence (CAPS) marker, 40% were of the A1 mating type and 60% were of the A2 mating type, with an A1 : A2 isolate ratio demonstrating the predominance of the A2 mating type each year of the survey.

late blight disease, virulent races, sexual reproduction, *R*-genes, potato resistance

DE GRUYTER OPEN

doi: 10.1515/sab-2017-0025
Received for publication on April 20, 2017
Accepted for publication on May 15, 2017

INTRODUCTION

Phytophthora infestans (Mont.) de Bary, the cause of late blight disease, is economically the most important pathogen of potatoes worldwide, including the Czech Republic. The disease has become a worldwide problem since its causal involvement in the Irish potato famine in the mid-19th century (B o u r k e , 1964). The incident initiated a period of potato breeding for late blight resistance. Nevertheless, the planting of resistant cultivars has not been the only part in the complex management of the disease. Although control of potato late blight is based on integrated disease management strategies (breeding, chemical protection, etc.), world economic losses are estimated at 12 billion EUR per year (H a v e r k o r t et al., 2009). Similarly, as in other crops, genetic improvement of late blight resistance has become an effective means of controlling late blight on potato, requiring not only functional genes, but also accurate knowledge of pathogen population structure. The key aspect in the development of a new resistant cultivar is to have a sufficient number of genes that are effective against the pathogen. In the past, the majority of genes associated with a hypersensitive reaction of potato cells infected with an avirulent race of *P. infestans* have been introgressed into the

* Supported. by the Ministry of Agriculture of the Czech Republic, Project No. QJ1210305.

cultivated potato (*Solanum tuberosum*) from *Solanum demissum*, the secondary gene pool species. At least 11 single resistance genes in loci *R1–R11* (Black et al., 1953; Malcolmson, Black, 1966) that control the hypersensitive response in the interaction between a resistance (*R*) gene (receptor) of the host and a corresponding avirulence (*Avr*) gene (elicitor) of the pathogen according to Flor's theory of host–pathogen relationship (Flor, 1971) and represent the basic gene pool of potato resistance to *P. infestans,* have been widely utilized in potato breeding. Mutations in genes controlling avirulent elicitors have led to differentiation of physiological races in the *P. infestans* population, and their selection can overcome the mechanism of hypersensitive response. It is for this reason why most genes have lost their effectiveness due to the race-specific nature of resistance and, thus, their breeding potential. To define pathogen virulence (physiological races, virulence factors, and virulence phenotypes), Black's (Scottish) and Mastenbroek's (Dutch) differential sets consisting of genotypes, each carrying a specific *R*-gene, were developed (van Poppel et al., 2009). Over the last two decades, the majority of resistance gene loci have been mapped, *R1* as the first locus (Ballvora et al., 2002) and *R9a* as the last locus (Jo, 2013). Considering the breakdown of potato resistance mediated by the matching *R*-gene, it is very important to acquire a genotype with a combination of several genes, as is known in potato cultivar (cv.) Sarpo Mira. Sarpo Mira contains a combination of four pyramided qualitative *R*-genes and a quantitative resistance gene, together providing a high level of field resistance (Rietman et al., 2012). The other two known genes that were successfully introgressed into *S. demissum* from the tertiary gene pool species *S. bulbocastanum* are *Rpi-blb-1* (van der Vossen et al., 2003) and *Rpi-blb-2* (Haverkort et al., 2009). Potato cvs Bionica and Toluca were the first to contain the *Rpi-blb-2* gene conferring a broad-spectrum resistance to *P. infestans* (Haverkort et al., 2009). Zhu et al. (2015) employed cvs Bionica and Toluca into sets of trap plants to monitor virulence in the *P. infestans* populations under field conditions. The virulence and race structure of *P. infestans* populations have been extensively monitored since the 1980s, alongside changes in pathogen populations relating to the possibility of sexual reproduction of *P. infestans.* For example, Andrivon (1994) compared the structure and dynamics of virulence within American and European pathogen populations collected over twenty-seven years. Flier et al. (2007) published a comprehensive study comparing virulence diversity in *P. infestans* isolates originating from four European countries, and other works presented local race composition of *P. infestans* in Western European (Pilet et al., 2005; Savazzini, Galetti, 2015), Nordic (Hermansen et al., 2000; Lehtinen et al., 2008), Baltic (Runno-Paurson et al., 2011; Aav et al., 2015), and Polish populations (Michalska et al., 2016). In all of these studies, survey data showed that there was complex virulence to several different *R*-genes in *P. infestans* populations. An increased level of virulence complexity has been accelerated by potato trade globalization and by sexual reproduction corresponding with the presence of two specialized mating strains, A1 and A2. The ability to reproduce sexually has led to increased occurrence of new recombinant genotypes including host-adapted and complex physiological races. In the Czech Republic, the occurrence of mating types of *P. infestans* has been systematically monitored since 2003 (Mazakova et al., 2006, 2010).

By contrast, although late blight is a limiting factor in potato production in the Czech Republic, the spatial and temporal distribution of physiological races of *P. infestans* in the country has not yet been documented. Therefore, this work presents the first complex study of virulence of the Czech *P. infestans* isolates and maps the occurrence of races virulent to important major *R*-genes derived from *S. demissum* and *S. bulbocastanum* and their stability in potato-growing areas of the Czech Republic from 2012 to 2014 and in 2016. Furthermore, the isolates collected through the survey were analyzed for mating type to determine country-wide and temporal changes in mating type distribution.

MATERIAL AND METHODS

Phytophthora infestans isolates

Over a period of 4 years (2012–2014 and 2016), 338 monosporic isolates (35 in 2012, 40 in 2013, 65 in 2014, and 203 in 2016) of *P. infestans* were collected from 31 sites, including gardens, small farms, and research stations in the two most important potato production regions (the Vysočina region and the Central Bohemia region) and in three other regions (the Pardubice region, the Plzeň region, and the Prague region) of the Czech Republic. Because the years of the survey may be characterized as atypical 'late blight years' due to unusual weather conditions, the geographical distribution of sampling sites and the mean number of isolates sampled per site varied from year to year depending on the occurrence of the pathogen in the potato crops. Therefore, potato leaves with typical late blight symptoms showing sporangiophores at the edge of water-soaked lesions on an abaxial leaf surface were collected randomly. Immediately after transport to the laboratory, leaflets with sporangiophores were placed upper surface down in a glass Petri dish equipped with filter paper moistened with double-distilled water and incubated overnight in the dark at 16–18°C. From a fresh, abundant sporulating lesion, single sporangia were collected by trapping sporangia on a small agar

piece stuck to the tip of a sterile injection needle and placed onto rye A medium (C a t e n , J i n k s , 1968). After incubation at 16–18°C in the dark, isolates were subcultured by transferring a mycelial plug from the colony edge onto a new growth medium.

Potato plant material

The differential set of potato clones included standard Black's differentials, each containing one of the *R1–R11* race-specific *R*-genes (except *R5*), the susceptible clone *R0* (GRIN IDs 07S0200398 to 07S0200408), cv. Sarpo Mira with *R3a*, *R3b*, *R4*, and *Rpi-Smira1* genes (GRIN ID 07S0102213), and somatic hybrid REG 46F (GRIN ID 07S0200439) with a *Rpi-blb-1* gene produced through protoplast electrofusion between a late blight-resistant *S. bulbocastanum* clone PI243510 and a diploid potato line 81.20/23 **(**GRIN ID 07S0500005) derived from cv. Apta (S e d l a k o v a , 2010). All clones were kindly provided by the Gene Bank of the Potato Research Institute Havlíčkův Brod, Ltd., Czech Republic. Plant materials of all potato genotypes were maintained *in vitro* by monthly subculturing on MS medium (M u r a s h i g e , S k o o g , 1962). The meristematic clones were transferred into perlite. After one week of cultivation, the clones were re-potted in soil for *ex vitro* acclimation of the plants and they were grown under greenhouse conditions.

Detached leaflet assays

The detached leaf assay was used to test the virulence of 142 isolates of *P. infestans*. The 4- to 6-week-old lateral leaflets were collected in the morning and individually placed abaxial side up in a humid chamber (a 60-mm-diameter polystyrene Petri dish equipped with double-distilled water-wetted filter papers). Three detached leaflets of each differential and the other clones were used for each isolate. An inoculum suspension for each isolate was prepared by harvesting sporangia from fresh pure cultures (subcultured only 2 or 3 times) in double-distilled water and by cooling at 4°C for 2 h. The spore concentration in the inoculation suspension was adjusted to 1.5×10^4 ml^{-1}. Two 20-µl drops of inoculum were placed on each leaflet. Inoculated leaflets were incubated in a cultivation chamber MLR-351H (Sanyo Electric Co., Ltd., Moriguchi, Japan) at 18°C in the dark for 24 h. They were then turned over and incubated at 18°C with a photoperiod of 16 h light and 8 h dark for the next 4 days. Compatible or incompatible interaction between a specific virulence factor and a specific *R*-gene was evaluated by assessing the sporulation observed on leaflets. Isolates sporulating only outside the inoculation zone were rated as virulent; those isolates that did not sporulate, but at least caused micro-necrosis, were rated as avirulent. A test was repeated with fresh inoculum if no sporulation was observed on susceptible clone (*R0*), and in that case, no micro-necrosis was visible on the other differential potato clones since immunity was not expected.

Mating type determination

The mating type of each *P. infestans* isolate was determined using the pairing test with reference isolates (A1 02 BASF 05 and A2 02 BASF 10) kindly provided by D.E.L. Cooke (SCRI, Dundee, UK) and the cleaved amplified polymorphic sequence (CAPS) marker using a W16 primer set and restriction enzyme *Hae*III syn. *Bsu*RI (J u d e l s o n et al., 1995). Both procedures are described in detail in M a z a k o v a et al. (2010).

Data analysis

To compare the frequencies of virulent races within the years of the survey, data obtained from a detached leaflet assay were analyzed using the chi-square test run in MS Excel 2010 software. The distribution of virulence in 2012 was set as the standard for the following years. To compare the frequencies of mating types within the years of the survey, Pearson's chi-square and Cramér's V tests were performed. Data were summarized into contingency tables and analyzed using STATISTICA version 12 CZ for MS Windows. A chi-square goodness of fit test was conducted to compare the observed ratio of mating types in each site with the expected theoretical ratio 1 : 1, indicating the possibility of sexual reproduction (STATISTICA). The significance level for all tests was set as $P < 0.05$.

RESULTS

Virulence tests

All 142 isolates tested were successfully evaluated for their virulence/avirulence to potato clones of Black's differential set, cv. Sarpo Mira and somatic hybrid REG 46F. Observed interactions resulted in symptoms ranging from micro-necrosis at the site of inoculation to a large area of sporulation (spots exceeding 50% of leaf area) outside the inoculation site. Generally, a high occurrence of isolates virulent on most differential clones was observed. With the exception of virulence for *R5*, which was not included in the differential set, and *R9*, which was not found in the isolates, all known virulence genes were detected among isolates collected during the survey. The most frequently overcome *R*-genes (generally more than 85% of records) were *R1* (98.6% isolates), *R3* (95.1%), *R7* (95.1%), *R10* (87.9%), and *R11* (90.8%). Frequencies ranging from 43 to 75% were found within isolates virulent to *R2* (61.3%), *R4* (74.6%), and

Table 1. Frequency of Czech *Phytophthora infestans* isolates virulent on potato clone differentials in the years 2012–2014 and 2016

Year	No. of isolates	*R1*	*R2*	*R3*	*R4*	*R6*	*R7*	*R8*	*R9*	*R10*	*R11*	Sblb	SM
2012	28	1.00	0.57	0.89	0.68	0.54	0.96	0.11	0.00	0.86	0.93	0.00	0.54
2013	38	1.00	0.68	1.00	0.71	0.61	1.00	0.00	0.00	0.95	0.92	0.00	0.76
2014	35	0.94	0.43	1.00	0.57	0.29	0.97	0.11	0.00	0.80	0.89	0.00	0.77
2016	41	1.00	0.73	0.90	0.98	0.32	0.88	0.02	0.00	0.90	0.93	0.00	0.41
Total	142	0.99	0.61	0.95	0.75	0.43	0.95	0.06	0.00	0.87	0.91	0.00	0.62

Sblb = somatic hybrid REG 46F with *Rpi-blb-1* gene, SM = cultivar Sarpo Mira

R6 (43%). Very low virulence frequencies were found to *R8* (5.6%). All isolates were virulent on reference clone *R0*. The frequency of isolates overcoming the gene complex of cv. Sarpo Mira was 62%. No isolate was found to be able to overcome *Rpi-blb-1*. A total of 38 different races was detected. Most isolates (96%) were virulent to five or more *R*-genes of potato clones simultaneously, with the average number of *R*-genes overcome (overall virulence complexity) of 7.1 (Fig. 1). The most common virulence phenotype was the complex race virulent to R1.2.3.4.6.7.10.11, followed by the complex race virulent to R1.2.3.4.7.10.11. No statistically significant year effect was detected for the frequencies of races ($P = 0.749$ in 2013, $P = 0.501$ in 2014, and $P = 0.18$ in 2016), indicating the high year-over-year stability of the occurrence of *P. infestans* races throughout the survey. The relatively higher year-over-year variation (20–40%) was observed in the occurrence of isolates virulent on clones carrying the genes *R2*, *R4*, *R6* and cv. Sarpo Mira (Table 1). The isolates virulent on clones bearing the genes *R1*, *R7*, *R10*, *R11* as well as cv. Sarpo Mira, were widely distributed at all sampling sites. The virulence to genes *R2, R3, R4,* and *R6* was detected in 73–93% of sampling sites, depending on the sampling year.

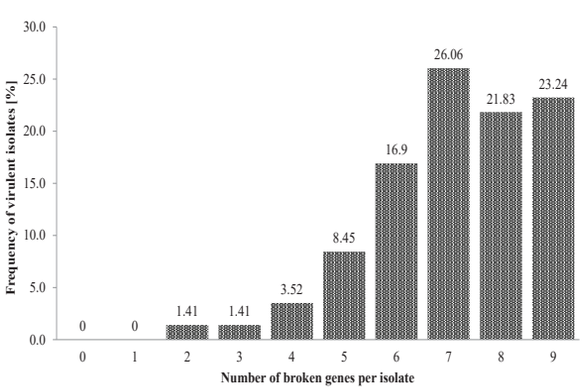

Fig. 1. Frequency of Czech *Phytophthora infestans* isolates by number of *R*-genes overcome (mean for the years 2012–14 and 2016), indicating high virulence complexity with mean value of 7.1

Isolates with the virulence gene corresponding to *R8* were recorded at five sites (Fig. 2).

Mating types

The pairing test and CAPS marker revealed with complete correspondence that all the Czech *P. infestans* isolates belonged to one of the mating types (Table 2). No self-fertile isolate was detected. Of the 338 isolates examined, 40% were of the A1 mating type and 60% were of the A2 mating type. The proportion of both mating types varied with the year in which the isolates were collected. The proportions of A2 mating type were higher in 2013 (60%), 2014 (73%), and 2016 (58%) than those of A1 mating type. In 2012, the proportion of A1 (51%) and A2 (49%) mating types was almost equal. There was no significant positive relationship between the frequencies of A1 and A2 mating types across years, with observed χ^2 values and *P*-values of 6.8 and 0.079, respectively. The distribution of mating types also varied considerably at each site. The proportion of A1 mating types ranged from 0 to 100% in 2012, 0 to 60% in 2013, 0 to 67% in 2014, and 0 to 97% in 2016, depending on a site where more than one isolate was collected from a field. The frequency of A2 mating type ranged from 0 to 100% each year of the survey (Table 2). A common occurrence of both mating types was observed at three sites sampled in 2012, 2013, and 2014 and four sites sampled in 2016. The ratio of mating types was not significantly different from 1 : 1 at only four sites, with χ^2 values and *P*-values ranging from 0 to 0.826 and from 0.363 to 1, respectively.

DISCUSSION

Information about the occurrence of virulence in *P. infestans* populations has been reported from countries in and outside Europe (e.g. Andrivon, 1994; Cooke et al., 2011; Runno-Paurson et al., 2011; Michalska et al., 2016). Nevertheless, in the Czech Republic, no data have been published about this important phenomenon influencing potato resistance to late blight. The results of the present

study, thus, provide the first comprehensive view of the race structure of *P. infestans* populations in the Czech Republic. At the same time, this study also adds new data about occurrence of mating types to previous findings (Mazakova et al., 2006, 2010). It is not known which races of *P. infestans* were present on the potatoes or whether changes in the virulence of *P. infestans* occurred prior to 2012 because the pathogen races were not previously monitored. However, the data obtained during this survey can be compared with those from other countries. The results of the detached leaflet assay showed that almost all (> 85%) isolates were virulent on potato clones with genes *R1*, *R3*, *R7*, *R10,* and *R11*. Our findings approximately correspond to other recent studies from the nearby geographical regions. In Poland, Michalska et al. (2016) observed a very high frequency (100%) of the same virulence factors as well as factor 4 in *P. infestans* isolates collected from 2005 to 2007. A virulence spectrum consistent with the aforementioned work has been presented in other previous studies from Poland (Sliwka et al., 2006; Chmielarz et al., 2014). In contrast, while Polish isolates virulent to *R8* were frequently present (68%), the Czech isolates were virulent to *R8* at a very low frequency (5.6%). Our results also closely correspond to those obtained by Aav et al. (2015), who found that more than 80% of isolates collected in Latvia from 2010 to 2012 were virulent to *R1*, *R3*, *R4*, *R7*, *R10,* and *R11*. Similarly, almost all *P. infestans* isolates collected in Estonia from 2001 to 2008 were virulent on potato clones carrying genes *R1*, *R3*, *R4*, *R7*, *R10,* and *R11* (Runno-Paurson et al., 2011). No isolate was sporulated on the potato clone with *R9*; we only observed a significant level of leaf area damaged in the inoculating zone, indicating a hypersensitive reaction. Isolates overcoming gene *R9* were found in Poland and Estonia, although the frequency of virulence to gene *R9* was very low (Sliwka et al., 2006; Runno-Paurson et al., 2011; Chmielarz et al., 2014; Michalska et al., 2016). In contrast, although the frequency of isolates virulent to this *R*-gene was the lowest (24%) among the other ones detected in Latvia, it was still somewhat higher when compared with results of other studies. Comparing the results presented here with results of virulence spectra in Nordic populations of *P. infestans*, isolates of *P. infestans* virulent to *R1*, *R3*, *R4*, *R7*, *R10,* and *R11* were predominant (≥ 90%) in populations in all four countries (Lehtinen et al., 2008). In our study, isolates collected in all years of the survey represented races with a high virulence complexity independent of isolate origin (cultivar, sampling locality, and time). We observed a high frequency (> 90%) of complex races expressing 5 or more virulent factors, with a predominant occurrence of virulence phenotype 1.2.3.4.6.7.10.11. The two most common virulence phenotypes in Poland were 1.2.3.4.5.6.7.8.10.11 and 1.2.3.4.5.6.7.10.11 (Michalska et al., 2016). In Latvia, the most frequent complex virulence was the 1.2.3.4.7.10.11 virulence phenotype, whereas virulence phenotype 1.3.4.7.10.11 was prevalent in Estonia (Runno-Paurson et al., 2011). The comparison of *P. infestans* isolates collected in the Czech Republic during the survey period shows high year-over-year

Table 2. Sampling sites and numbers of A1 and A2 mating types in Czech *Phytophthora infestans* isolates in the years 2012–2014 and 2016

Year/sampling site	Region and district code[1]	Number of tested isolates		
		A1	A2	ΣA1 + A2
2012 (*n* = 35 isolates)				
Valečov (Okrouhlice)	CZ0631	4	6	10
Olešnice (Okrouhlice)	CZ0631	0	3	3
Lučice	CZ0631	2	2	4
Malčín	CZ0631	0	3	3
Frýdnava	CZ0631	3	0	3
Veselý Žďár	CZ0631	2	0	2
Jedouchov (Věž)	CZ0631	0	1	1
Kamenice (Heralec)	CZ0631	1	2	3
Bystřec	CZ0534	6	0	6
2013 (*n* = 40 isolates)				
Valečov (Okrouhlice)	CZ0631	9	6	15
Veselý Žďár	CZ0631	4	4	8
Český Dvůr (Knyk)	CZ0631	0	4	4
Rozsochatec	CZ0631	3	8	11
Čachotín	CZ0631	0	2	2
2014 (*n* = 65 isolates)				
Veselý Žďár	CZ0631	10	5	15
Valečov (Okrouhlice)	CZ0631	5	6	11
Pohled	CZ0631	0	1	1
Modlíkov	CZ0631	0	3	3
Nové Dvory	CZ0631	0	3	3
Semice	CZ0208	0	20	20
Čelákovice	CZ0209	1	6	7
2016 (*n* = 203 isolates)				
Malý Bor	CZ0322	0	29	29
Domanínek	CZ0635	15	18	33
Praha-Suchdol	CZ0100	3	0	3
Lukavec	CZ0633	1	12	13
Želiv	CZ0633	1	13	14
Lípa	CZ0631	0	33	33
Valečov (Okrouhlice)	CZ0632	59	2	61
Veselý Žďár	CZ0633	1	0	1
Velhartice	CZ0322	0	10	10
Únětice	CZ020A	6[t]	0	6[t]

n = number of collected isolates, t = isolates collected from tomatoes

[1]region and district code is based on NUTS classification

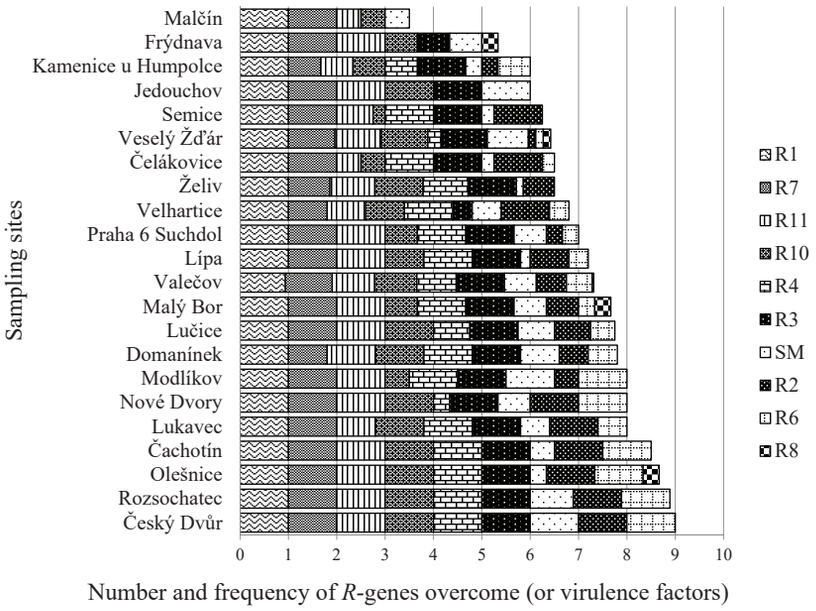

Fig. 2. Frequency of Czech *Phytophthora infestans* isolates virulent on potato clone differentials at selected sampling sites (2012–14 and 2016)

The colour of the columns represents *R*-genes overcome by isolates. The length of the columns represents the frequency of virulence of *P. infestans* isolates at given sampling sites. For example, all (100%) isolates sampled in Malčín were virulent on two potato clone differentials (*R1*, *R7*) and 50% of isolates sampled in Malčín were virulent on three potato clone differentials (*R10*, *R11*, SM). SM = cultivar Sarpo Mira

stability of virulence to major *R*-genes. Genetic stability in populations of *P. infestans* and distribution of virulence factors are still a key aspect for sustainability of resistance breeding, namely for selecting effective genetic resources and using resistant cultivars with accumulated resistance genes. *P. infestans* is highly adaptable and its success depends on the genotypic variation in the population; thus, deployment of major *R*-genes from *S. demissum* in potato breeding does not seem to be a sustainable approach for the management of late blight over the long term. However, despite the rapid breakdown of most *R*-genes in the past, *S. demissum* is still considered a valuable source for both race-specific and race-non-specific resistance and there are still efforts to find effective and durable *R*-genes (Fry, 2008). In addition, other wild *Solanum* spp. such as *S. bulbocastanum* are also being considered as possible sources of resistance (Haverkort et al., 2009). Therefore, the knowledge of resistance potential of genes derived from *S. bulbocastanum* is of interest for breeders. This species could be incorporated into breeding programs because genes from this germplasm confer broad spectrum resistance to *P. infestans*.

The control of late blight using genes conferring resistance to disease is further complicated by sexual reproduction of the pathogen. Coexistence of both mating types in the same field indicates a potential of sexual reproduction which increases genotypic variation and thereby the rate of pathogen adaptation (Cooke, Lee, 2004). Until the 1980s, the common presence of both mating types had been known only in Mexico (Niederhauser, 1991). Since then, the A2 mating type has been detected in almost all countries of the world, including Europe (e.g. Sliwka et al., 2006; Lehtinen et al., 2008; Cooke et al., 2011; Runno-Paurson et al., 2011). In the Czech Republic, the A2 mating type was first observed in 2003 and has been subsequently found in other years (Mazakova et al., 2006, 2010). In this study, both mating types were present at thirteen sites in which more than one isolate was collected, indicating a potential for sexual recombination. However, the ratio of both mating types was significantly different from 1 : 1 at nine sites; so the formation of oospores could be limited. The proportion of the A2 mating type was higher than that of the A1 mating type during three years of the survey. This finding is consistent with the results of a previous study performed in the Czech Republic from 2007 to 2008 (Mazakova et al., 2010) and in 2009 (unpublished data). By contrast, isolates of the A1 mating type were predominant in 2003, 2005 (Mazakova et al., 2006), and 2006 (unpublished data). The current prevalence of the A2 mating type is likely due to the stable flow of potato propagation material (seed) and a decrease in the potato-growing area, which limits the migration and instability of the *P. infestans* populations. A comparison of the results obtained in this study with those previously reported in other countries of the European region showed that the A1 : A2 ratio is regionally specific. Despite this finding, recent studies have shown a higher proportion of the A1 mating type in Latvia (Aav et al., 2015), Estonia (Runno-Parson et al., 2011), and Poland (Chmielarz et al., 2014). As noted by Hermansen

et al. (2000) and Cooke, Lee (2004), a comparison of results of regional studies may be difficult and inappropriate because the provided data are often based on different numbers of isolates and monitoring sites.

CONCLUSION

The data presented in this study showed that the structure of the Czech *P. infestans* populations is relatively stable. Nevertheless, *P. infestans* is considered to be a well-adapted pathogen with a high evolutionary potential and, thus, future detailed studies including genotypic characterization may reveal that the population structure of *P. infestans* is variable and different from that presented here.

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