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Changes in specific virulence in Polish populations of *Phytophthora infestans*: 1985–1991

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Abstract

Ninety-five isolates of *Phytophthora infestans* collected throughout Poland during 1985–1991 and characterized for multilocus genotypes based on mating type, allozymes and DNA fingerprint, were analyzed for specific virulence to differential potato cultivars carrying ten major resistance genes. The multilocus analysis led to three groupings. The first group contained 22 isolates of a clonal lineage (PO-1) that is postulated to have been present in Europe during most of the twentieth century, but PO-1 isolates were recovered in Poland only during 1985–1988. This group contained, on average, virulence to 5.5 specific resistance genes per isolate. The second group consisted of 30 isolates in a clonal lineage (PO-4) that had not been detected before 1988. PO-4 isolates had virulence to a mean of 6.5 resistance genes per isolate. The third group was composed of 43 isolates representing 38 multilocus genotypes also not detected before 1988. These diverse genotypes had virulence to an average of 6.7 specific resistance genes per isolate. More than half (53%) of the PO-4 isolates shared a single pathotype. The group of 43 isolates was dominated by two pathotypes: the most common one (47% of the isolates) was the same pathotype that dominated PO-4 isolates; the next most common one (21%) differed from the most common one by the absence of virulence to resistance gene R5. The recent immigrant isolates (not detected before 1988) generally had virulence to a greater number of specific resistance genes than did isolates in the previous population [detected before 1988 (PO-1)]. Recent immigrant populations were dominated by one or two pathotypes, so their pathotypic diversity values were somewhat less than that of the previous population.

Introduction

Prior to the availability of biochemical and molecular technologies, specific virulence was one of the major characteristics used to distinguish one isolate of a plant pathogenic fungus from another. However, when a new race appeared, investigators could not know whether it was a mutation from an indigenous genotype, or whether it was a recent immigrant that was unrelated to individuals in the previous population. Biochemical and molecular technologies such as allozymes, nuclear and mitochondrial DNA restriction fragment length polymorphisms, DNA fingerprinting and Random Amplified Polymorphic DNAs (RAPDs) have enabled dramatic increases in our understanding of the population genetics of fungal plant pathogens (McDermott and McDonald, 1993; McDonald and McDermott, 1993).

Use of molecular and biochemical technologies in population studies of the potato late blight fungus, *Phytophthora infestans* (Mont.) de Bary, has revealed dramatic changes recently in populations of this organism in Poland (Spielman et al., 1991; Sujkowski et al., 1994) and all over the world (Fry et al., 1993; Goodwin et al., 1994a, 1994b, 1995a; Koh et al., 1994). These changes were first signalled by the discovery of iso-

lates with the A2 mating type, initially in Switzerland (Hohl and Iselin, 1984), and then in many locations (Fry et al., 1993). Although variation for molecular markers has been studied intensively, the specific virulence characteristics of the immigrant populations are largely unknown. Recent studies in the Netherlands and the United States suggest that the current immigrant populations there are more complex (individuals contain a greater number of specific virulence factors) than were the previous indigenous populations (Drenth et al., 1994; Goodwin et al., 1995b).

The migration of new genotypes into Poland and the subsequent displacement of the old population by the migrating population began in the late 1980s (Spielman et al., 1991; Sujkowski et al., 1994). From 1985–1987, all isolates collected in Poland represented the PO-1 clonal lineage, also been termed 'US-1' (Goodwin et al., 1994a, b) and 'old' (Drenth et al., 1994). This clonal lineage is postulated to have existed in Europe for many years, possibly since the 1840s (Goodwin et al., 1994b; Sujkowski et al., 1994). The PO-1 clonal lineage was not detected after 1988 (Sujkowski et al., 1994). Genetic diversity within Polish populations of *P. infestans* increased greatly during the late 1980s, with the immigration of many new genotypes. One of these clonal lineages, PO-4, predominated in the late 1980s and early 1990s, with a frequency of about 0.3 (Sujkowski et al., 1994). Some of these new migrants were of the A2 mating type and sexual recombination apparently now affects the genetic structure of *P. infestans* populations in Poland (Sujkowski et al., 1994).

Because of the dependence in Poland on resistant cultivars to manage late blight, specific virulence in Polish populations of *P. infestans* is of significant practical importance. The first virulence analysis in *P. infestans* isolates collected at the Potato Research Institute at Mlochow was reported by Pietkiewicz (1978). Most isolates in this study were fairly complex, with a mean number of virulences of around four per isolate. Unfortunately, the number of resistance genes tested was not consistent from year to year, and ranged from four (in 1971) to nine (in 1973–1974), so the results of this earlier study are not directly comparable to results using a more complete set of differential cultivars. Isolates of the earlier study are no longer available, but they presumably represented PO-1.

The purpose of the present study was to investigate differences in virulence between isolates representing the old clonal lineage of *P. infestans* (PO-1) that was present in Poland before 1988 compared to the new immigrant genotypes. If the number of pathotypes within a lineage increases with time due to mutations (Goodwin et al., 1995b), then individuals of the PO-1 lineage collected in the late 1980s should constitute a more diverse population for pathotype than would individuals of a clonal lineage that has recently arrived in Poland (e.g., PO-4) that has had less time in which to accumulate mutations.

Materials and methods

Sources of isolates

Ninety-five isolates of *Phytophthora infestans* were collected during 1985–1991 in fields of commercial potatoes and from advanced breeding lines at agricultural research stations throughout Poland and analyzed for virulence to ten potato resistance genes. Details of the collection sites are provided elsewhere (Sujkowski, 1992; Sujkowski et al., 1994). These 95 isolates have been characterized for allozyme genotypes at the *glucose-6-phosphate isomerase* and *Peptidase* loci and for DNA fingerprints using probe RG57 (Sujkowski et al., 1994).

Virulence testing

The specific virulence of each isolate was determined using detached leaflets. Differential cultivars containing resistance genes R1–R5, and R7–R11 had been obtained from the Scottish Crop Research Institute, Dundee, UK and from the Instituut voor Planteziektenkundig Onderzoek, Wageningen, the Netherlands. The plants were produced from virus-free tissue culture stocks maintained at Mlochow, Poland and were grown subsequently in a greenhouse. Additional illumination from high-density sodium lights was provided as necessary to maintain a 12- hr photoperiod. Five leaflets were collected from the middle part of each differential cultivar at 6–8 weeks of age. Leaflets were placed in moist chambers abaxial surface up, and inoculated with a suspension of a standardized concentration of sporangia ($0.5–1.5 \times 10^5$ sporangia mL^{-1}). The sporangia were obtained from cultures that had grown on Rye A agar (Caten and Jinks, 1968) for 10–14 days at 16–20 °C. Two drops (ca. 50 microliters each) of the sporangial suspension were placed on each leaflet, one on each side of the midrib.

The interactions between the fungus and potato genotypes were scored after six days of incubation at 18 °C. Each test included the susceptible cultivar Tarpan (containing no known R-genes) as a control.

Only data from tests in which the fungus produced large, profusely sporulating lesions on Tarpan were used. Compatibility was defined as the ability of an isolate to produce large, profusely sporulating lesions on at least three of the five leaflets. An interaction was scored incompatible if fewer than three leaflets had large, profusely sporulating lesions. To minimize the effect of environmental variation, all inoculations were performed in a limited time from the end of September until the end of October each year (1985–1991). Compatible interactions were usually indicated by large, sporulating lesions on all five inoculated leaflets. In contrast, incompatible interactions typically were characterized by no sporulation on any of the leaflets, and in many cases there was only a massive hypersensitive reaction. The majority of virulence assays were repeated at least twice.

Differences in diversity of pathotypes within lineages were quantified using the Shannon information statistic (Sheldon, 1969) normalized as described elsewhere (Goodwin et al., 1993). Values for the normalized index range from 0.0 (when all individuals are identical) to 1.0 (when all individuals are different from each other).

Results

Analysis of recent individuals representing the historical clonal lineage, PO-1, indicated a rather large number of specific virulence factors (Table 1) with a mean of 5.5 factors per isolate. The most frequently occurring specific virulence factors were virulence to resistance genes R1 (frequency = 0.96), R4 (frequency = 0.91), and R11 (frequency = 0.83) (Table 2). Virulence to R9 was not detected and virulence to R8 was rare. Frequencies of the remaining five factors ranged from 0.26 to 0.78 (Table 2). There were 12 pathotypes containing from two to nine virulence factors among the 22 isolates (Table 1).

The average number of virulence factors in the new predominating clonal lineage PO-4 (6.5) was higher by one than the average virulence complexity of PO-1 (5.5) (Table 2). However, the pathotype diversity was greater in PO-1 than in PO-4 (Tables 1 and 3). The Shannon diversity index of pathotypes for the PO-4 clonal lineage (n = 30) was 0.5 versus 0.7 for PO-1 (n = 22) (Table 3). Lineage PO-4 was dominated (frequency = 0.5) by a single highly complex pathotype (1,2,3,4,7,10,11) (Table 1), that was present in different locations all over the country (Figure 1). In contrast, lineage PO-1 was not dominated by any particular pathotype (Table 1). In the PO-4 lineage, frequencies of virulence to resistance genes R2, R3 and R7 were much higher than in PO-1 (Table 2). However, PO-1 had one isolate with the highest virulence complexity (1,2,3,4,5,7,8,10,11) found in Poland (Table 1).

For comparison, a group of additional isolates (n = 43) representing 38 multilocus genotypes was assessed for specific compatibility factors. Twenty of these isolates shared the pathotype 1,2,3,4,7,10,11, which was also the most common pathotype in PO-4. Nine additional isolates shared pathotype 1,2,3,4,5,7,10,11, (Table 1). Both pathotypes were detected in many locations throughout Poland (Figures 1, 2), but detections of pathotype 1,2,3,4,5,7,10,11 in lineage PO-4 were concentrated in eastern Poland (Figure 2). Generally, the frequency of specific virulence factors in the group

Table 1. Number of isolates of each pathotype in the PO-1 and PO-4 clonal lineages[1] and in a group of 38 distinct genotypes of *Phytophthora infestans* collected in Poland during 1985–1991

Pathotype	Clonal lineage group		
	PO-1	PO-4	38 genotypes
7,11	1	0	0
1,3,11	0	1	0
1,4,11	2	0	0
1,3,4,7	0	0	1
1,3,4,11	0	0	1
1,2,3,11	1	0	0
1,4,10,11	5	0	0
3,4,7,10	0	1	0
1,2,3,4,7	1	0	4
1,2,3,4,10	1	1	0
1,2,3,4,5,10	1	0	0
1,2,3,4,7,10	0	1	2
1,2,3,4,7,11	0	3	3
1,3,4,10,11	1	0	0
1,3,4,7,10,11	0		0
1,2,4,5,10,11	1	0	0
1,2,3,7,10,11	0	1	0
1,2,3,4,10,11	0	0	1
1,2,3,4,5,7,11	0	0	2
1,2,3,4,7,10,11	4	16	20
1,2,3,4,5,7,10,11	3	3	9
1,2,3,4,5,7,8,10,11	1	0	0
Sample size	22	30	43

[1] These lineages are described in Sujkowski et al. (1994); PO-1 is identical to the US-1 genotype described by Goodwin et al. (1994b) and 'old' as described by Drenth et al. (1994).

Table 2. Frequencies of virulence in the PO-1 and PO-4 clonal lineages[1] of *Phytophthora infestans* and in the group of 38 genotypes in Poland during 1985–1991

Virulence to resistance gene	PO-1 (N = 22)	PO-4 (N = 30)	38 genotypes (N = 43)
R1	0.96	0.97	1.00
R2	0.61	0.83	0.95
R3	0.61	1.00	1.00
R4	0.91	0.93	1.00
R5	0.26	0.10	0.26
R7	0.48	0.93	0.95
R8	0.04	0.00	0.00
R9	0.00	0.00	0.00
R10	0.78	0.87	0.74
R11	0.83	0.90	0.84
Mean number of virulences/isolate	5.45	6.53	6.74

[1] Described in Sujkowski et al. (1994); PO-1 is identical to the US-1 genotype described by Goodwin et al. (1994b) and 'old' as described by Drenth et al. (1994).

Table 3. Normalized Shannon's diversity indices[1] for pathotype of *Phytophthora* infestans within clonal groupings (including a group of distinct genotypes) and by year in Poland during 1985–1991

Grouping	Shannon's diversity index	Number of isolates
By clonal grouping (all years)		
PO-1 clonal lineage	0.73	22
PO-4 clonal lineage	0.47	30
38 distinct genotypes	0.44	43
By year (all lineages)		
1985–1987	0.64	11
1988	0.40	23
1989	0.74	15
1991	0.37	46

[1] As described in Goodwin et al. (1993).

of 43 isolates was slightly higher than that in the isolates representing the PO-4 clonal lineage (Table 2).

There were differences in pathotypic diversity over the years of study as measured by the normalized Shannon's diversity index (Table 3). The highest diversity values were observed in 1987 (0.64) and 1989 (0.74) and the lowest occurred in 1991 (0.37) (Table 3).

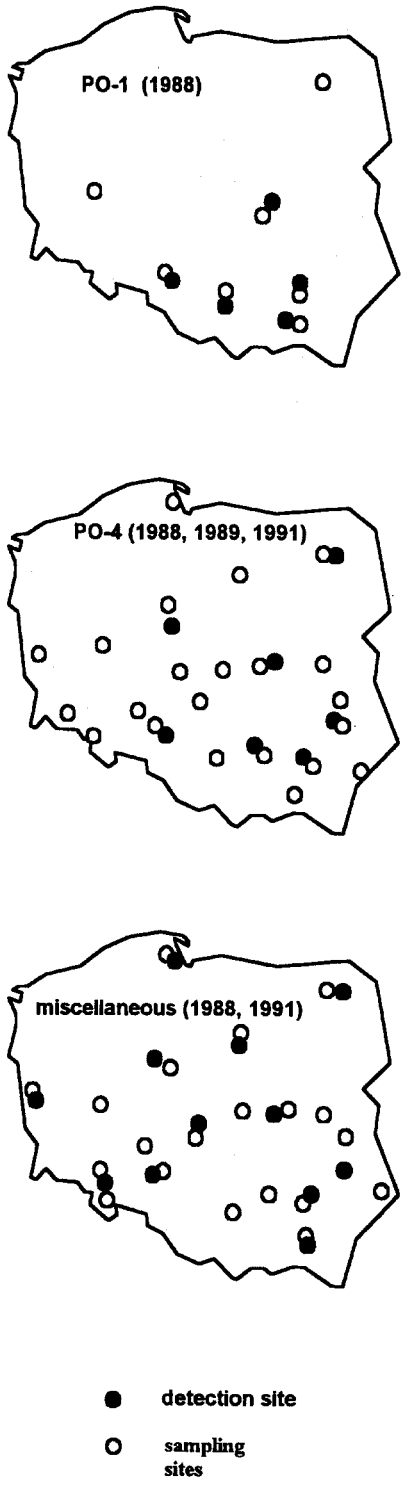

Figure 1. Distribution of the dominating pathotype (1,2,3,4,7,10,11) of *Phytophthora infestans* over lineages, genotypes and regions in Poland, 1988–1991.

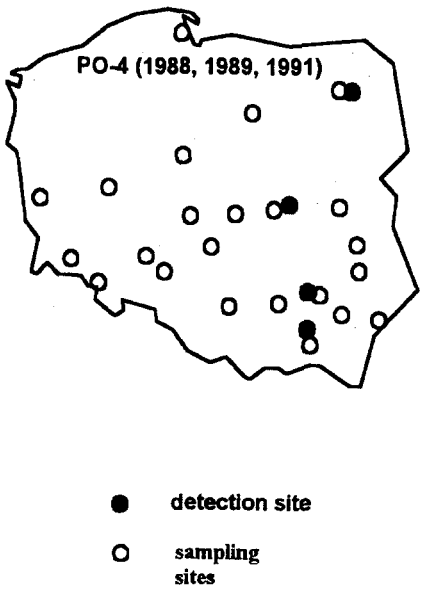

Figure 2. Distribution of pathotype 1,2,3,4,5,7,10,11 of *Phytophthora* infestans in clonal lineage PO-4 in 1988, 1989, and 1991.

Discussion

Results of this investigation illustrate considerable pathotypic diversity and complexity in Polish populations of *P. infestans*. They also support the initial hypothesis that individuals of the clonal lineage present in Poland for a longer time (PO-1) were more diverse (contained a greater number of specific pathotypes) than a clonal lineage (PO-4) present in Poland for a shorter time. Isolates of PO-1 even had higher diversity than a group of 38 distinct multilocus genotypes identified with molecular markers (Table 3). Genetic drift and selection may have lowered the virulence diversity in founder populations of new genotypes. Virulence appears to evolve very rapidly (Goodwin et al., 1995b), and the PO-1 clonal lineage has had more time to accumulate virulence mutations compared to populations founded by recently immigrated genotypes.

The mechanism for pathotypic differences between lineages is not known for certain. The old clonal lineage in Poland was displaced by new immigrating genotypes very quickly (Spielman et al., 1991; Sujkowski et al., 1994). Therefore, mutations during the time of displacement seem unlikely to have contributed to the recently observed pathogenicity increases and changes in virulence architecture. Instead, it seems probable that genetic drift and selection associated with the founding of new populations by immigration are the major factors. Founder effects have also been proposed to explain differences in virulence frequencies among years in France and Wales (Andrivon, 1994).

Two lines of evidence indicate that the observed increase in specific virulence was a result of migration. First, a greater number of specific virulences per isolate was detected after the immigration than before. We found that the average number of specific virulences per isolate was 6.8 after 1988 (after migration) but was 4.2 in isolates collected before 1988. Second, a large number of pathotypes in the new population were able to overcome resistances of the cultivar Bronka and advanced breeding lines of the Polish potato breeding program. Bronka and the advanced selections have unidentified major gene(s) (Swiezynski et al., 1991). Prior to 1988 Bronka was only blighted in a field trial in the Toluca Valley in central Mexico (T. M. Sieczka, personal communication) where most isolates contain a large number of specific virulence factors (Tooley et al., 1986, Rivera-Peña, 1990). No pathotype of the P0-1 clonal lineage was able to produce sporulating lesions on Bronka either in the laboratory or in the field trials (L. S. Sujkowski, unpublished results). Therefore, the current late blight international differential set may need to be augmented by one or more cultivars containing resistance genes from Bronka to fully characterize virulence diversity in the immigrating populations of *P. infestans* in Europe.

Several specific virulence or avirulence factors were near fixation in Polish populations of *P. infestans*. Specific virulence against resistance genes R1, R3, and R7 occurred in more than 90% of the new isolates. Avirulence to resistance gene R9 was fixed in the total collection, and only one isolate out of 95 analyzed in our study appeared to be compatible to resistance gene R8. The decrease in pathotypic diversity in 1991 was due to the predominance of a singly highly complex pathotype, and this also affected the frequencies of the individual virulence factors.

It seems highly likely that the amount of pathotypic diversity found within the old clonal lineage (PO-1) in Poland might have resulted from the accumulation of mutations. This lineage in the Netherlands, Peru and the United States was dominated by a few relatively simple pathotypes (Drenth et al., 1994; Goodwin et al., 1995b; Tooley et al., 1985), although highly complex pathotypes were found occasionally (Goodwin et al., 1995b). This lineage has existed in Poland for

many years, possibly more than a century (Goodwin et al., 1994b). Extensive use of resistance genes from *Solanum demissum* particularly R1–R4, in the Polish breeding programs (Swiezynski et al., 1991) since the 1940s (Schick and Klinkowski, 1962) and subsequent adoption of the resulting cultivars (Sieczka, 1979; Swiezynski et al., 1993) probably created strong selection pressure for virulent mutants that may have helped increase the pathotypic diversity within this lineage.

Lack of detailed information on the specific R-genes in Polish cultivars and on the area on which they are planted precludes a direct analysis of the potential role of selection. However, detection of pathotypes as complex as 1,2,3,4,5,7,8,10,11 in the mid 1980s compared to less complex pathotypes detected in earlier studies (Pietkiewicz, 1978) suggests that selection might have been important in the evolution of virulence. Pathotype 1,2,3,4,7,10,11 was the second most commonly detected pathotype among PO-1 isolates and predominated in PO-4 and the other new genotypes (Table 1). The occurrence of a single complex pathotype in many different lineages and in most years suggests that it may have some sort of selective advantage. However, because we do not know if the corresponding combination of resistance genes occurs in commonly grown Polish potato cultivars, the exact basis for the possible selective advantage remains uncertain.

Because complex races occurred commonly prior to the immigration of new genotypes, there was little opportunity in Poland for a large increase in specific virulence between the 1980s and 1990s. This is different from the situation in the Netherlands and in the USA where large increases in specific virulence occurred as a result of immigration (Drenth et al., 1994; Goodwin et al. 1995b). Polish populations of *P. infestans* were similar to those in eastern Germany. East German populations contained an average of 5.1 virulences per isolate in 1981 and 7.1 in 1985 (data from Andrivon (1994)), remarkably close to the corresponding values in Poland. This may indicate a greatest similarity among populations of *P infestans* in eastern Germany and Poland compared to other areas in Europe.

The increases in pathotype complexity probably represent additional difficulties for potato late blight management in Poland. Prior to these migrations, resistant cultivars had contributed importantly to a total late blight management program. The subsequent appearance of additional specific virulence factors and combinations (via immigration) and their probable rearrangement into new genotypes (via sexual reproduction) may explain the decreased resistance in cultivars like Bronka. Bronka had been extremely resistant to late blight before the old population was displaced in Poland, but has been more severely diseased in the late 1980s and early 1990s (L. S. Sujkowski, unpublished observations). Future plant breeding efforts probably need to concentrate on more durable (polygenic) resistances.

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