Phenotypic Changes in Populations of *Phytophthora infestans* from Eastern Germany

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ABSTRACT

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A total of 106 isolates of *Phytophthora infestans* collected in eastern Germany between 1976 and 1990 was analyzed for mating type, allozyme phenotype, and response to metalaxyl. The A2 mating type was found among isolates collected in 1980. Although an increase in the frequency of the A2 mating type has been observed since 1976, it did not increase to a frequency of 0.5, which is found in Mexico. Metalaxyl resistance was first observed among isolates collected in 1977, prior to the commercial use of the fungicide in eastern Germany. Between 1976 and 1990 the frequency of isolates resistant within a sample to metalaxyl increased from 0 to 40%. The number of isolates within a sample exhibiting an intermediate response to metalaxyl fluctuated from year to year. Prior to 1984 all A1 isolates, except one, had the dilocus allozyme phenotype *Gpi-1* (glucose phosphate isomerase) 86/100, *Pep-1* (peptidase) 92/100. A2 mating type isolates were 100/100 for *Gpi-1* and either 100/100 or 92/100 for *Pep-1*. The predominant dilocus phenotype for isolates collected from 1984 to 1988 was 100/100 for both *Gpi-1* and *Pep-1*. Two new phenotypes, *Pep-1* 83/100 and *Gpi-1* 90/100, appeared in the population in 1987 and 1990, respectively. By 1990, the frequency of *Pep-1* 83/100 isolates had increased to 26%. The remainder of the 1990 isolates were 100/100 for *Pep-1*. Eighty-eight percent of the isolates collected in 1990 were 100/100 for *Gpi-1*. The remaining 12% were 90/100. The changes in phenotypes observed in the eastern German population were similar to those being observed elsewhere.

Phytophthora infestans (Mont.) de Bary, the causal agent of late blight of potato (*Solanum tuberosum* L.) and other solanaceous plants, is a heterothallic fungus that reproduces sexually by means of two mating types, designated A1 and A2. In 1984, the A2 mating type was reported among specimens collected in Switzerland during 1981 (16). This was the first published report of the existence of the A2 mating type outside the Toluca Valley in Mexico, where both mating types exist in approximately equal proportions and where sexual reproduction appears to be widespread (10,34). Since 1981, the A2 mating type has been found in most of the potato-growing regions of the world in which late blight occurs, including the United Kingdom (17), Germany (11,24) Poland (19), Israel (13), Japan (18,32), and the Commonwealth of Independent States (formerly the Union of Soviet Socialist Republics) (12). Inasmuch as no culture collections contain A2 isolates collected prior to 1980, it has been hypothesized that the A2 mating type may have been introduced to countries outside Mexico at about that time (27). An alternative explanation is that the A2 mating type had been present, yet went undetected (26). Furthermore, the frequency of A2 mating type isolates appears to have increased in western Germany (24) and Poland (22) during the 1980s, and it is the predominant mating type in Israel (13) and Japan (32). Conversely, an increase in frequency of the A2 mating type does not seem to have occurred in the United Kingdom (26) and the Netherlands (33) during the same time period.

Resistance to the systemic fungicide metalaxyl among field isolates of *P. infestans* initially was reported in Ireland (7) and the Netherlands during 1981 (5). The resistant phenotype has now been reported among many populations of *P. infestans* (2,11, 21,22,24,26). An increase in the frequency of metalaxyl resistance has been observed in Israel (3), Northern Ireland (4), the United Kingdom (26), and Poland (23). With the presence of the A2 mating type in Europe, as well as the increase in occurrence of metalaxyl resistance, concern that sexual recombination may occur and generate progeny of increased pathogenicity and less sensitivity to metalaxyl has risen.

The goal of this investigation was to determine whether phenotypic changes, similar to those found elsewhere (28,29), have occurred among populations of *P. infestans* in eastern Germany (formerly the German Democratic Republic). Thus, isolates collected from 1976 to 1990 were analyzed for mating type, response to metalaxyl, and allozyme phenotype.

MATERIALS AND METHODS

Source of isolates. Isolates were collected from potato-growing regions of eastern Germany from 1976 to 1990 (Fig. 1 and Table 1). Blight infested potato leaves were collected at random, and *P. infestens* was isolated into pure culture using standard techniques (14). Isolates were maintained using paraffin oil and Henniger medium, a semiartificial medium consisting of agar, amino acids, and dry potato powder (15), at the Institute for Potato Research (Gross Lüsewitz, Germany). Isolates were transferred to Rye A agar (1) prior to importation into the United States under a permit from the U. S. Department of Agriculture, Animal Plant Health Inspection Service. Cultures were maintained on Rye A agar slants at 8 C, and all laboratory manipulations of living cultures were conducted in a class II, Type A, laminar-flow biohazard hood.

Mating type determination. Mating type was determined by pairing eastern German isolates individually with Mexican isolates of known mating type. The pairings were made by inoculating the unknown isolates with Mexican tester isolates on 20% V8 agar in 100- × 15-mm petri dishes and by incubating the crosses in the dark at 18 C for 10–14 days. If oospores were observed microscopically at the hyphal interface between the unknown isolate and the Mexican isolate 519 (A2 mating type), the unknown isolate was designated A1. The unknown isolate was designated A2 if oospores were formed in pairings with Mexican isolate 560 (A1 mating type). All mating type assays were performed twice.

Metalaxyl response. Response to metalaxyl was determined by inoculating 4- × 4-mm hyphal plugs of *P. infestans* isolates

onto 20% V8-agar medium amended with 1 ml of technical-grade metalaxyl (gift from Ciba-Geigy Corp., Greensboro, NC) dissolved at appropriate concentrations in dimethylsulfoxide (DMSO). One milliliter of the metalaxyl/DMSO solution was added to autoclaved culture media to create a final fungicide concentration of 5 μg/L and 100 μg/L. For an unamended control, 1 ml of DMSO alone was added to the sterilized medium prior to its being dispensed into 100- × 14-mm petri dishes. Cultures were incubated at 18 C in the dark. Based on the percentage of hyphal growth relative to the control, individual isolates were classified as being sensitive (S), intermediate (I), or resistant (R), in their response to metalaxyl: S = growth less than 40% of control on both 5 μg/L and 100 μg/L; I = growth 40% or more of control on 5 μg/L, but less than 40% of control on 100 μg/L; and R = growth 40% or more of control on 100 μg/L. The cut-off points came from parallel assays, using this method and the floating leaf method described by Davidse et al (5). All metalaxyl response assays were performed twice.

Allozyme phenotype. Isolates were grown in pea broth for 21 days at 18 C, and the mycelium was harvested by filtration. Pea broth was prepared by sterilizing 125 g of peas for 15 min in distilled water. Peas were removed by pouring the mixture through a Buchner funnel. Distilled water was added to the filtrate to create 1 L of broth, which was dispensed into four 500-ml flasks, was sterilized, and was inoculated with two 8- × 3-mm agar plugs of *P. infestans*. Protein was extracted by adding 1 ml of homogenization buffer (1.21 g of Tris, 0.292 g of EDTA, 38 mg of NADP, and 1 L of water; pH 6.8) to the mycelium and homogenizing the mixture using a Brinkman Polytron. Samples were centrifuged at 10,000 rpm for 20 min. The supernatant was collected in disposable culture tubes and was stored at −80 C for later use. Two protein extractions were carried out for each isolate.

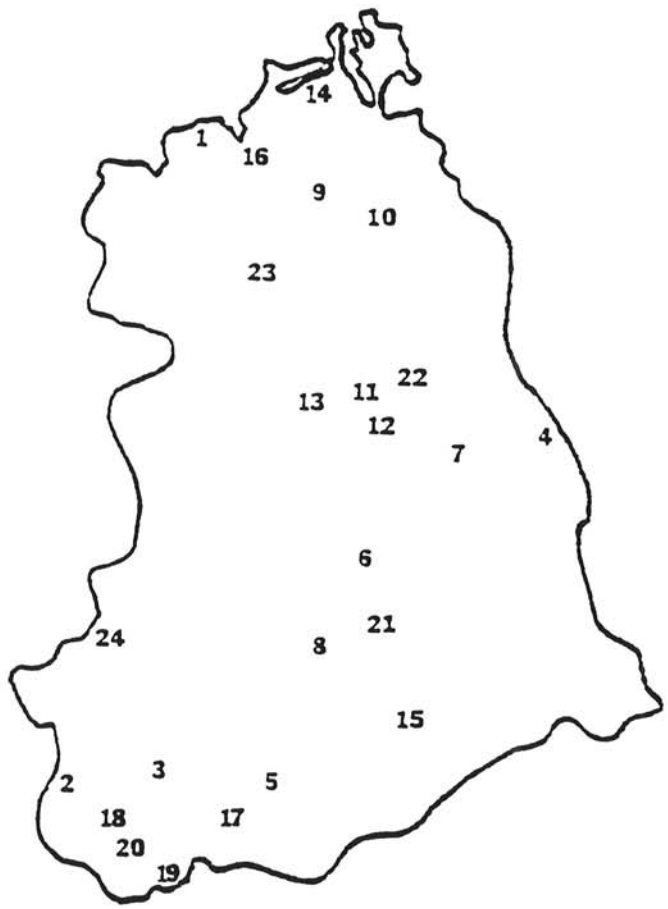

Fig. 1. Locations in eastern Germany (formerly the German Democratic Republic) where the 106 isolates of *Phytophthora infestans* used in this study were collected. Collection sites are numbered with corresponding names listed in Table 1.

Multilocus enzyme electrophoresis using the horizontal starch gel technique (25,34) was performed at least twice on the homogenates. Scoring of allozyme phenotypes is based on the relative mobilities of the protein bands from the cathode in the starch gels (34). The most common band is designated as 100 and the other bands are assigned numbers based on their mobilities relative to the 100 band. An isolate with a band that migrates 90% as far as the most common band would be designated as having a 90/100 phenotype. Two polymorphic isoenzymes (allozymes) have been adequately resolved for scoring and used in a number

TABLE 1. Collection sites, collection years, and the accession numbers of isolates of *Phytophthora infestans* collected in eastern Germany from 1976 to 1990

Collection site	Years	Accession number[a]
1. Bad Doberan	1985	51
	1988	29
2. Bad Salzungen	1989	33
3. Erfurt	1987	25
4. Frankfurt/Oder	1990	35X, 36X
5. Gera	1987	26
6. Jessen	1987	22
7. Konigs-Woesterhausen	1981	14
	1989	35, 56
8. Leipzig	1990	27X, 28X, 29X, 30X, 48X
9. Malchin	1988	49
10. Neubrandenburg	1990	46X, 47X
11. Neuruppin	1988	32, 34
12. Potsdam	1990	31X, 32X, 33X, 34X, 37X, 38X, 39X, 40X 42X, 43X
13. Rathenow	1988	28
14. Ribniz-Damgarten	1989	45, 46
15. Rochlitz	1987	24
16. Rostock	1981	7
	1984	16, 19
	1986	20
	1987	21
	1988	52, 53, 54, 55
	1989	43, 44, 47, 48, 57, 60, 63, 65, 66, 67,
	1990	1X, 2X, 5X, 44X, 45X, 54X, 67X, 68X, 69X, 71X, 72X, 73X, 74X, 75X, 76X, 77X, 80X, 81X, 82X
17. Schleiz	1985	18
18. Schmalkalden	1988	30
19. Sonneberg	1986	27, 58, 59, 62, 64
20. Suhl	1988	31
	1990	55X, 56X
21. Torgau	1987	23
22. Wentow (Grausee)	1977	1, 2, 3, 10, 11
23. Wittstock	1985	17
	1989	12, 36, 37, 38, 39, 40, 41, 42
24. Worbis	1981	50 (A2)

[a] No record of the collecting sites exists for isolates 4,5,6,8,9 (all collected in 1976), 13, and 15 (both 1980 A2 isolates). All A2 isolates are underlined.

TABLE 2. Mating types (A1 and A2)[a] of *Phytophthora infestans* and response to metalaxyl among German isolates collected between 1976–1990

Year	Number of German isolates	A1			A2		
		S	I	R	S	I	R
1976	5	4	1	0	0	0	0
1977	5	0	4	1	0	0	0
1980	2	0	0	0	0	1	1
1981	3	0	0	1	1	1	0
1984	2	2	0	0	0	0	0
1985	3	0	0	0	1	2	0
1986	6	1	0	0	4	1	0
1987	6	2	2	2	0	0	0
1988	11	4	0	6	1	0	0
1989	23	9	2	4	5	2	1
1990	40	13	6	16	4	1	0

[a] Metalaxyl response: S = sensitive, I = intermediate, R = resistant.

of previous genetic studies of *P. infestans* (30,33–35): glucose phosphate isomerase (EC 5.3.1.9.) (*Gpi-1*) and peptidase (EC 3.4.3.1.) (*Pep-1*). For *Gpi-1*, the gel buffer was 0.01 M histidine-HCl, pH 6.0, and the electrode buffer was Tris-citrate, pH 6.0 (30). The gel buffer for *Pep-1* was Tris-citrate, pH 6.7, and the electrode was Tris-citrate, pH 6.3 (25).

All 106 isolates were resolved for *Pep-1*; only 105 were scored for *Gpi-1*. Controls used to assess the relative mobilities of the allozymes were Mexican isolates 533, 550, and 560 (32) and Polish isolates 1142, 1168, and 1182. Isolate 1182 has the 90 and 83 alleles for *Gpi-1* and *Pep-1*, respectively (L. J. Spielman, *unpublished data*). Because questions exist as to the ploidy of the eastern German isolates, and therefore the genotypic information obtained from scoring the gels for these allozymes, the term "phenotype" will be used when referring to allozyme designations for the eastern German isolates.

RESULTS

Mating type determination. The A2 mating type was not present among isolates collected in 1976 and 1977. However, 26 of the isolates collected between 1980 and 1990 were mating type A2 (Table 2). Sample size was small and varied from year to year, so the results of this survey make it difficult to conclude that an increase in A2 mating type frequency has occurred in eastern Germany as it has occurred elsewhere in Europe (22,24). The ratio of the two mating types does not approach the 1:1 ratio found in samples from Mexico (10,34). Because no oospores appeared outside the hyphal interface between the unknown and the tester isolates, it is unlikely that any of the eastern German isolates are self-fertile.

Metalaxyl response. Responses to metalaxyl are shown in Table 2. A single isolate that showed an intermediate level of metalaxyl resistance was found among the isolates collected in 1976. The first resistant isolate was found in the 1977 collection. Between 1976 and 1990 the number of isolates per sample resistant to metalaxyl increased from 0 to 40%. The majority of resistant isolates were mating type A1. When analyzed by Chi-square, a significant correlation was found between the A1 mating type and metalaxyl resistance ($\chi^2 = 6.92$, $P < 0.05$). For both A1 and A2 mating types, the frequency of isolates displaying intermediate resistance fluctuated from year to year.

Allozyme phenotype. Allozyme phenotypes are shown in Tables 3 and 4. Nine of the 10 pre-1980 isolates had the dilocus phenotype combination *Gpi-1* 86/100, *Pep-1* 92/100 (Table 4). The 100/100 phenotype first appeared for *Gpi-1* and *Pep-1* in 1980. Since 1980, this has become the predominant phenotypic combination among European populations. Two new alleles, *Pep-1* 83 (1987) and *Gpi-1* 90 (1990), appeared among the eastern German isolates. Of the isolates collected in 1990, 35 out of 40 were 100/100 for *Gpi-1* (Table 5). The remaining 5 were 90/100 for *Gpi-1*. The 1990 isolates were predominantly 100/100 for *Pep-1*; however, 11 of the 1990 isolates displayed the 83/100 phenotype.

TABLE 3. Accession numbers, mating types, and allozyme phenotypes for *Gpi-1* and *Pep-1* of *Phytophthora infestans*

Accession number	Mating type	*Gpi-1*	*Pep-1*
1,2,3,4,5,6,7,8,9,10	A1	86/100	92/100
45	A1	86/100	100/100
11	A1	122/100	92/100
31,53,37,41,48,56	A1	100/100	92/100
47×	A1	90/100	83/100
40×,44×,45×	A1	90/100	100/100
60,66	A1	100/100	92/92
26,42,31×,32×,33×,34×, 46×,55×,56×,72×,77×	A1	100/100	83/100
16,19,20,21,22,23,24,25, 28,29,30,32,33,34,40,46, 49,52,54,57,63,65,67,1× 2×,5×,27×,28×,29×,30×, 35×,37×,38×,39×,42×,43×, 48×,54×,68×,69×,71×, 74×,75×,80×,81×	A1	100/100	100/100
12,14,15,17,18,27,35,36, 38,39,43,44,50,51,55,58, 59,36×,67×,76×	A2	100/100[a]	100/100
13	A2	100/100	92/100
47,73×	A2	100/100	83/100
62	A2	100/100	92/92
82×	A2	90/100	100/100

[a] Isolate 64, an unlisted A2, had no activity for *Gpi-1*, but was 100/100 for *Pep-1*.

DISCUSSION

Since the initial report of the *P. infestans* A2 mating type among isolates collected from potato in Switzerland in 1981 (14), the A2 mating type has been found in populations from both European and non-European countries (6,8,11,13,18,22,24,26,30). However, with the exception of the 1989 Polish populations (32), the two mating types have not been found in a 1:1 ratio outside the central highlands of Mexico. A 1:1 ratio of both mating types in a population normally results in an interbreeding sexual population (33), as has been found in Mexico (28,34)

The A2 mating type does not appear to have been present in eastern Germany prior to 1980 (Table 2). It is noteworthy that the two 1980 A2 isolates from eastern Germany predate the earliest reported collection of the A2 mating type from outside Mexico (16,31,33). Our observation that the A2 mating type does not occur among isolates collected prior to 1980 supports the hypothesis that the A2 mating type was first introduced into Europe in, about, 1980. An alternate hypothesis, that the A2 mating type was present but undetected for many years (24), does not seem to be borne out by independent observations of the appearance of the A2 mating type at about the same time in

TABLE 4. Allozyme phenotypes of *Gpi-1* and *Pep-1*, the number of German isolates of *Phytophthora infestans* demonstrating each for the 1976–1990 period

	Number of *P. infestans* isolates							
	Gpi-1 phenotypes[a]				*Pep-1* phenotypes			
Year	86/100	90/100	100/100	122/100	83/100	92/100	92/92	100/100
1976	5	0	0	0	0	5	0	0
1977	4	0	0	1	0	5	0	0
1980	0	0	2	0	0	1	0	1
1981	1	0	2	0	0	1	0	2
1984	0	0	2	0	0	0	0	2
1985	0	0	3	0	0	0	0	3
1986	0	0	5	0	0	0	1	5
1987	0	0	6	0	1	0	0	5
1988	0	0	11	0	0	2	0	9
1989	1	0	22	0	2	4	2	15
1990	0	5	35	0	11	0	0	29

[a] One isolate demonstrated no activity for *Gpi-1*.

TABLE 5. Phenotype frequencies in collections of *Phytophthora infestans* from eastern Germany

Gpi-1	*Pep-1*	Mating type	Collection dates				
			1976–86	1987	1988	1989	1990
Old phenotypes[a]							
86/100	92/100	A1	0.40	0	0	0	0
86/100	100/100	A1	0	0	0	0.04	0
122/100	92/100	A1	0.04	0	0.18	0.17	0
100/100	92/100	A1	0	0	0	0	0
		A2	0.04	0	0	0	0
100/100	92/92	A1	0	0	0	0.09	0
		A2	0	0	0	0.04	0
New phenotypes							
90/100	83/100	A1	0	0	0	0	0.02
90/100	100/100	A1	0	0	0	0	0.08
		A2	0	0	0	0	0.02
100/100	83/100	A1	0	0.17	0	0.04	0.22
		A2	0	0	0	0.04	0.02
100/100	100/100	A1	0.12	0.83	0.73	0.30	0.56
		A2	0.40	0	0.30	0.30	0.08
Sample size			25[b]	6	11	23	40

[a] Old phenotypes are those that are found in higher frequency among isolates collected in the 1970s and early 1980s. New phenotypes have only been found within samples collected since the early 1980s (28,29).
[b] One isolate demonstrated no activity for *Gpi-1*.

a number of European countries (16,17,11,22,24) or by its complete absence among isolates collected prior to 1980. Although it is generally true that the A2 mating type has not shown an overall increase in frequency in most European countries since its initial discovery, it did increase in frequency in Poland between 1987 and 1989 (23) and in the Federal Republic of Germany between 1985 and 1987 (24).

Our observations, and those of others (24,26,32), that the two mating types do not occur in a 1:1 ratio in most European populations of *P. infestans* does not exclude the possibility that sexual reproduction occurs in the European populations. In fact, oospores were found on blighted plant material collected from potato fields near Cottbus, Germany (11).

In our study, the allozyme phenotypes were similar to those observed in earlier analyses of isolates from Mexico (34), Peru (35), Japan (32), the Netherlands (9), England and Wales (26), Poland (29), and Ireland (P. W. Tooley et al, *unpublished data*). All isolates that were of the *Gpi-1* 86/100, *Pep-1* 92/100 phenotype were mating type A1. There was a noticeable change in the phenotypes during the 15-yr sampling period (Table 5). Although most isolates collected since 1980 have been 100/100 for both *Gpi-1* and *Pep-1*, the increasing presence of the *Gpi-1* 90 and *Pep-1* 83 alleles is similar to that which has been observed in the Netherlands (9), Poland (29), and Ireland (P. W. Tooley et al, *unpublished data*). The variation in allozyme phenotype for the two enzymes used in this investigation demonstrated that phenotypic changes have occurred within the eastern German population of *P. infestans*. However, because of the small number of samples and the diversity in sampling locations, a quantitative determination of these changes cannot be made (19,20). The phenotypic changes observed in the eastern German population are similar to those recently demonstrated in other European populations, including the Netherlands (9), Poland (29), and Ireland (P. W. Tooley et al, *unpublished data*). Spielman et al (29) hypothesized that the extant European population is rapidly being displaced by a new population that has a different genotypic structure. The data from this study are consistent with their hypothesis.

Our discovery of a 1977 isolate that demonstrated partial metalaxyl resistance suggests that the resistance allele existed in the natural population prior to the beginning of commercial use of metalaxyl in eastern Germany in 1979. Afterward, the frequency of the resistance allele apparently increased in the A1 mating type isolates as a result of the selection pressures for fungicide resistance. This may explain the observed correlation between the A1 mating type and the resistance allele. A similar association between the A1 mating type and metalaxyl resistance had been previously reported in the population of *P. infestans* from Poland (23). It is probable that more judicious use of metalaxyl since the reported occurrence of resistance in 1981 has limited an increase of the resistance allele among A2 isolates. The existence of the resistance allele in the natural population prior to the introduction of metalaxyl poses some interesting questions regarding other possible roles for this allele.

Although it appears that the A2 mating type has not increased in frequency in eastern Germany to the extent that it has in other European populations (23,24), changes in the phenotypic frequencies of *Gpi-1* and *Pep-1* have occurred that coincide with the appearance of the A2 compatibility type. It is not possible, however, to determine if sexual reproduction has been the driving force for the observed changes in the eastern German population of *P. infestans*. Some phenotypic changes have also been detected that do not coincide with the appearance of the A2 mating type. Mutation or migration are the most likely forces behind these changes (9,29).

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