Widespread Distribution and Probable Origin of Resistance to Metalaxyl in Clonal Genotypes of *Phytophthora infestans* in the United States and Western Canada

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ABSTRACT

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The sensitivity of 251 isolates of *Phytophthora infestans* to the phenylamide fungicide metalaxyl was assessed by an in vitro radial growth assay on fungicide-amended agar media. Isolates were collected from many regions of North America from 1987 through 1993, including 15 states in the United States and British Columbia, Canada. A small sample of isolates from Europe and Israel was included for comparison. Isolates that grew less than 40% of the control on media containing 5 μg of metalaxyl per ml were considered sensitive; all other isolates were scored as resistant. Field trials and floating leaf-disk assays were used to confirm the accuracy of the amended-agar technique. All isolates collected from 1987 through 1989 were sensitive. Metalaxyl-resistant isolates were detected in 13 of 15 states and in British Columbia during 1992 and 1993. With one exception, sensitivity to metalaxyl was absolutely correlated with clonal lineage as determined by mating type, allozyme genotype, and DNA fingerprint analysis. The US-1 clonal lineage, present in the United States and Canada for many years, was uniformly sensitive. In contrast, all isolates with the recently immigrated US-7 and US-8 clonal genotypes were resistant, even those obtained from fields with no history of metalaxyl application. All US-6 isolates collected since 1990 were resistant, but one sensitive US-6 isolate was collected in California in 1989. The cause of this polymorphism within US-6 could not be determined. Metalaxyl resistance was unimodally distributed within clonal lineages and limited to those that were recently immigrated. This strongly supports the hypothesis that resistance in the United States and Canada originated by migration, rather than by mutation and selection after migration. In contrast, evidence for selection of metalaxyl-resistant mutants within clonal lineages was detected among the limited sample of isolates from Europe and Israel.

The phenylamide fungicide metalaxyl (Ridomil) is highly effective in controlling disease caused by sensitive isolates of the potato and tomato late blight pathogen *Phytophthora infestans* (Mont.) de Bary. Unfortunately, resistance to this compound developed in populations of *P. infestans* in Ireland, the United Kingdom, and western Europe soon after it was introduced in the early 1980s (3,11,14,28). Metalaxyl resistance currently is widespread in Europe (3,7,10,11,14,15,28,34), the Middle East (6), Asia (29,30,39), Mexico (32), and Canada and the United States (12,13). Most locations sampled contain both sensitive and resistant individuals (12,15,29,30,32).

Previous analyses of genetic diversity within and among populations of *P. infestans* in the United States and Canada identified a limited number of clonal genotypes (21,27), only four of which were common during 1992 and 1993 (27). Among the four most common genotypes (designated US-1, US-6, US-7, and US-8 [21, 27]), one (US-1) is believed to have been present for many years, possibly since the 1840s (22). The other three (US-6, US-7, and US-8) are probably recent immigrants from northwestern Mexico (21,27). The US-6 genotype was most likely introduced during 1979; it was definitely present in California by 1982 (21). US-7 and US-8 both have the *111* allele at the allozyme locus *glucose-6-phosphate isomerase* (*Gpi*). This allele has not been reported outside of North America and was first found in northern Mexico in 1989 (26). Individuals containing this allele (the US-7 and US-8 genotypes) were probably introduced into the United States and Canada during or shortly before 1992 (27).

There are at least two potential origins for metalaxyl resistance in *P. infestans* in the United States and Canada. The first is that it was introduced by migration. The frequency of resistance to metalaxyl in northwestern Mexico was almost 100% by 1989 (32). Because US-7 and US-8 were probably introduced after 1989 (27), they were most likely already resistant at the time of migration. US-6 was introduced prior to metalaxyl use (21), so the initial

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migrants were probably sensitive. However, resistant isolates of US-6 were collected in northwestern Mexico during 1989 (26,32), so resistance in this genotype could have been introduced in a later migration. Thus, it seems highly likely that metalaxyl resistance was introduced and has spread throughout the United States with the migration of new genotypes. The alternative hypothesis is that resistance arose by mutation and selection within the United States. This could have occurred in previously existing clonal lineages or in new clonal lineages after their migration. Because resistance appeared so rapidly in other parts of the world, it seems likely that it could have evolved rapidly also in the United States and Canada.

Each hypothesis makes testable predictions about the patterns of metalaxyl resistance that should occur within populations. If resistance originated by migration, it should be limited to recently immigrated clonal lineages. Furthermore, because the frequency of resistance in the probable source population was near 100%, rare sensitive individuals within the immigrant lineages probably would have been eliminated by genetic drift (bottlenecks) during migration. Therefore, these lineages should be uniformly resistant, regardless of whether they came from sprayed or unsprayed fields.

TABLE 1. Sources of the 251 isolates of *Phytophthora infestans* from the United States and western Canada assayed for metalaxyl sensitivity

Location	Original host	Year	Sample size	Genotype(s)
British Columbia	Potato	1992	14	US-6, BC-1–BC-4
California	Tomato	1989	1	US-6
		1993	7	US-7
Florida	Potato	1993	6	US-6
	Tomato	1993	7	US-7
Idaho	Tomato	1993	1	US-9
Kentucky	Tomato	1993	1	US-7
Maine	Potato	1987	1	US-5
		1992	9	US-1
		1993	7	US-7, US-8
Michigan	Tomato	1993	3	US-7
Minnesota	Tomato	1993	1	US-7
New Jersey	Tomato	1992	1	US-1
New York	Potato	1987	29	US-1
		1992	66	US-7, US-8
	Tomato	1992	30	US-1, US-7
		1993	15	US-7
North Carolina	Tomato	1992	10	US-7
North Dakota	Potato	1992	7	US-1
Oregon	Potato	1992	14	US-1, US-6
Tennessee	Tomato	1992	2	US-7
Washington	Potato	1992	9	US-1, US-6
Wisconsin	Potato	1993	9	US-1, US-7, US-10

Analysis of resistance within a clonal lineage should give a unimodal distribution of either resistant or sensitive individuals. In contrast, if metalaxyl resistance was selected within the United States or Canada, it could have developed within both older and more recently immigrated clonal lineages. Populations in different fields could be 100% resistant, 100% sensitive, or polymorphic for metalaxyl sensitivity depending on the selection pressure that was applied. There also should be polymorphism for metalaxyl sensitivity within clonal lineages, with a higher frequency of resistance in fields that were sprayed compared to unsprayed fields. Analysis of metalaxyl resistance within a clonal lineage should give a bimodal distribution of resistant and sensitive individuals.

The purpose of this paper was to analyze the modality and levels of resistance to metalaxyl within clonal lineages of *P. infestans* in the United States and Canada, to test the hypothesis that metalaxyl resistance in these populations originated by migration from northwestern Mexico. Similar analyses were performed on small samples from Europe and Israel to test whether migration or mutation was the origin of metalaxyl resistance in other countries. A secondary goal was to compare results of the amended-agar assay with a floating leaf-disk assay and to validate these in vitro measurements with metalaxyl resistance in vivo by field experiments.

MATERIALS AND METHODS

Sources of isolates. Isolates for this study were chosen randomly from among 565 isolates collected previously in the United States and Canada (21,27) and stored in the culture collection at Cornell University, Ithaca, NY. Most of the isolates were collected during 1992 and 1993 (Table 1), but some were collected beginning in 1987. All isolates were characterized previously for mating type and genotype at the two allozyme loci, *Gpi* and *peptidase* (*Pep*) (21,27). A subset of isolates also was characterized for genotype at more than 25 loci revealed by the DNA fingerprint probe RG57 (23). Isolates that were identical for all markers were considered to have the same clonal genotype. Isolates that were identical except for a single change at one of the allozyme or DNA fingerprint loci were considered to belong to the same clonal lineage (21). In total, 251 isolates from 15 states and the Canadian province of British Columbia were included (Table 1). From 1 to 29 isolates were analyzed from each of the approximately 70 fields sampled, for an average of 3.6 isolates per field. These isolates belonged to 11 clonal genotypes (Table 2), only 4 of which were common (Table 2). The remaining lineages were less common, and some were represented by single isolates.

TABLE 2. Metalaxyl sensitivity according to clonal genotype of 251 isolates of *Phytophthora infestans* collected in the United States and western Canada during 1987 to 1993

Genotype[a]	Sample size	Mating type	Allozyme genotype		DNA fingerprint[d]	Mean metalaxyl response[e]		Classification[f]
			Gpi[b]	*Pep*[c]		5 µg/ml	100 µg/ml	
US-1	65	A1	*86/100*	*92/100*	10111010110011010000110011	9.7	8.3	S
US-5	1	A1	*100/100*	*92/100*	10111010010011010111110011	15.0	10.0	S
US-6	30	A1	*100/100*	*92/100*	10111110010011000010110011	82.5	58.3	R
US-7	98	A2	*100/111*	*100/100*	10011000010011010101110011	97.8	68.8	R
US-8	49	A2	*100/111/122*	*100/100*	10011000010011010000110111	62.3	32.9	R
US-9	1	A1	*100/100*	*83/100*	...[g]	80.0	37.0	R
US-10	1	A2	*111/122*	*100/100*	...	31.0	33.0	S
BC-1	3	A2	*100/111*	*100/100*	10000000010011010000110011	50.0	7.0	R
BC-2	1	A2	*100/100*	*100/100*	10001100000011010000110011	2.0	1.0	S
BC-3	1	A2	*100/100*	*100/100*	10100010010011000010110011	25.0	0.0	S
BC-4	1	A2	*100/100*	*100/100*	10000000000011000010110011	36.0	0.0	S

[a] As described previously (21,27).
[b] *Glucose-6-phosphate isomerase.*
[c] *Peptidase.*
[d] Presence (1) or absence (0) of RG57 fingerprint bands 1 through 25 (23) are indicated from left to right.
[e] Mean percent growth relative to control at two concentrations of metalaxyl for all isolates with each genotype.
[f] S = sensitive; R = resistant.
[g] Not determined.

For comparison with the United States and Canada, a small sample of isolates from Europe and Israel that had been collected during the early 1980s (22) was analyzed by clonal genotype to obtain information about the probable origin of metalaxyl resistance in those countries in which it was first detected.

Metalaxyl assays. A radial growth assay on metalaxyl-amended agar (7,32) was the primary method for estimating metalaxyl sensitivity, because it is operationally easier than the floating leaf-disk method (32). Isolates were plated onto 10% unclarified V8-juice (33) or rye B (4) agar amended with 5 or 100 µg of metalaxyl per ml. Rye B agar was used preferentially in later trials, because some isolates did not grow well on V8-juice agar. Metalaxyl was prepared as a 100 mg/ml stock solution in pure dimethylsulfoxide (DMSO) and was added to molten (50°C) agar after autoclaving. Each treatment had the same amount of DMSO. Plates were inoculated with 8-mm plugs taken from the borders of actively growing colonies. Metalaxyl sensitivity was scored after 7 days or (for slow-growing isolates) when the colony diameters of the controls were at least 25 mm. Colony size was estimated by taking the mean of two diameters 90 degrees apart through the center of each inoculum plug and were corrected by subtracting 8 mm for the diameter of the plug. There were two replications for each treatment. The mean colony diameters at 5 and 100 µg of metalaxyl per ml were divided by the mean colony diameter of the same isolates on the unamended control plates. Isolates that grew more than 40% of the control on 5 µg/ml metalaxyl medium were considered resistant. This concentration of fungicide provided excellent resolution of resistant and sensitive isolates in previous studies (10,30,32).

Floating leaf-disk assays (13,32) on a subset of 21 isolates were used to confirm the reliability of the agar assays. Leaf disks (14 mm diameter) of susceptible potato cv. Norchip were floated abaxial side up in water containing 0, 5, or 100 µg of metalaxyl per ml. There were five leaf disks per treatment, and each treatment contained an equal amount of DMSO. Leaf disks were inoculated with sporangial suspensions made from 10- to 14-day-old agar cultures. Sporangia also were collected occasionally from sporulating lesions or from sporulating tuber slices. The sporangia were collected in distilled water, chilled at 4°C for 30 to 90 min to induce zoospore formation, and placed onto leaf disks in approximately 50-µl droplets. Inoculated leaf disks were incubated under lights at 18°C for 5 to 7 (usually 6) days. The percentage of each leaf disk covered with sporulation was estimated visually with the aid of a dissecting microscope. Metalaxyl sensitivity was calculated as the percent leaf area covered with sporulation in the presence of 5 and 100 µg of metalaxyl per ml relative to the control. Sensitive isolates generally did not sporulate at all on 5 µg/ml metalaxyl medium.

Field experiments. To test whether isolates scored as resistant in vitro were resistant in vivo, field experiments were conducted at the Homer C. Thompson Research Farm of Cornell University in Freeville, NY (approximately 10 miles from Ithaca), during 1993 and 1994. Seed pieces of potato cv. Norchip were planted during the first week of June in plots that were 4 m long × 4 rows wide. Spacing between rows was 0.9 m, and spacing between seed pieces within the row was 0.23 m. Fertilizer (330 kg each of N, P, and K per ha) was applied at planting. Plots were cultivated and hilled during the second week of July. Herbicide (linuron at recommended rates) was applied after planting, but before emergence. Insecticides to suppress potato leaf hoppers and Colorado potato beetles were applied as needed.

Plots were inoculated with a single isolate of *P. infestans* during late July each year. A typical US-6 isolate was tested during 1993 (Washington isolate Pasco 92-2, which grew 99 and 91% of the control on 5 and 100 µg of metalaxyl per ml, respectively), and a US-8 isolate was tested during 1994 (Maine isolate ME-2A, growth 57 and 26% of the control on 5 and 100 µg of metalaxyl per ml, respectively). Inoculum consisted of sporangia washed from 2-week-old cultures of *P. infestans* grown on rye A agar (4). Approximately 50 ml of a sporangial suspension (1,500 sporangia per ml in 1993 and 1,000 sporangia per ml in 1994) was sprayed onto a single plant in the center of each plot. Inoculations were made on 28 and 29 July and 1 August 1993 and on 27 and 28 July 1994. Conditions favorable to late blight were achieved by periodic sprinkler irrigations as needed during the experiment. Irrigations usually were initiated in the early evening, so the leaves remained wet all night, and were applied at the rate of 0.25 cm/h for 1 to 2 h.

Several fungicide treatments were applied for 5 weeks, beginning after the initial lesions were visible, but before they began to sporulate. During 1993, some lesions appeared from neighboring plots, and thus, a few lesions in some plots had begun to sporulate prior to the initiation of fungicide applications. Three fungicides (mancozeb [Dithane M-45], metalaxyl [Ridomil 2E], and metalaxyl + mancozeb [Ridomil MZ58]) were applied at about 375 liters/ha with a hydraulic sprayer at approximately 9 kg/cm^2 at three (1994) or four (1993) rates (Table 3). The mancozeb treatments were applied each week, and the metalaxyl treatments were applied every other week. The metalaxyl + mancozeb applications were made every other week; the same amount of mancozeb as used the previous week was applied on the alternate weeks. There were four replications of each fungicide treatment in a completely randomized design, and unsprayed plots were used as controls.

The severity of disease (total amount of tissue infected or destroyed) was assessed every 3 to 5 days until the end of the season, using a modification of a Commonwealth Mycological Institute key (17). Analyses were performed on percent disease at a particular time during the season or on the areas under the disease progress curve (36).

RESULTS

Based on our criterion for metalaxyl resistance (growth at 5 µg/ml greater than 40% of the control) (10,32), resistant isolates were detected in 13 of 15 states (all except New Jersey and North Dakota) and British Columbia during 1992 and 1993. The floating leaf-disk assays confirmed the reliability of the agar assays, as reported by other investigators (13,32). Isolates that were rated sensitive on the agar assay also were sensitive in the leaf-disk assay and generally did not grow or sporulate at all on leaf disks floating on 5 µg/ml metalaxyl solution. The only exception was a British Columbia isolate with a potentially recombinant genotype (27) that was rated sensitive based on the agar assay but was rated resistant based on the leaf-disk assay. Some isolates grew very slowly on the agar assay and were scored as resistant but did not sporulate on floating leaf disks treated with metalaxyl. When retested on agar, these isolates were confirmed to be sensitive. Thus, the amended-agar assay occasionally gave inconsistent results when the isolates grew slowly. However, if the isolates produced a colony diameter greater than 25 mm in 7 to 10 days, the assays were accurate and repeatable. Isolates that grew slowly in the amended-agar assay and for which reproducible data could not be obtained were excluded from further analysis.

TABLE 3. Amount of fungicide (pounds of active ingredient per acre) applied per treatment (unsprayed plots were used as controls)

Treatment level	Fungicide		
	Dithane (M-45)[a]	Ridomil (2E)[b]	Ridomil (MZ58)[c]
1	0.96	0.20	0.96 + 0.20
2	0.48	0.10	0.48 + 0.10
3	0.24	0.05	0.24 + 0.05
4[d]	0.12	0.025	0.12 + 0.025

[a] Mancozeb alone.
[b] Metalaxyl alone.
[c] Mancozeb (first amount) + metalaxyl (second amount).
[d] This level was only used during 1993.

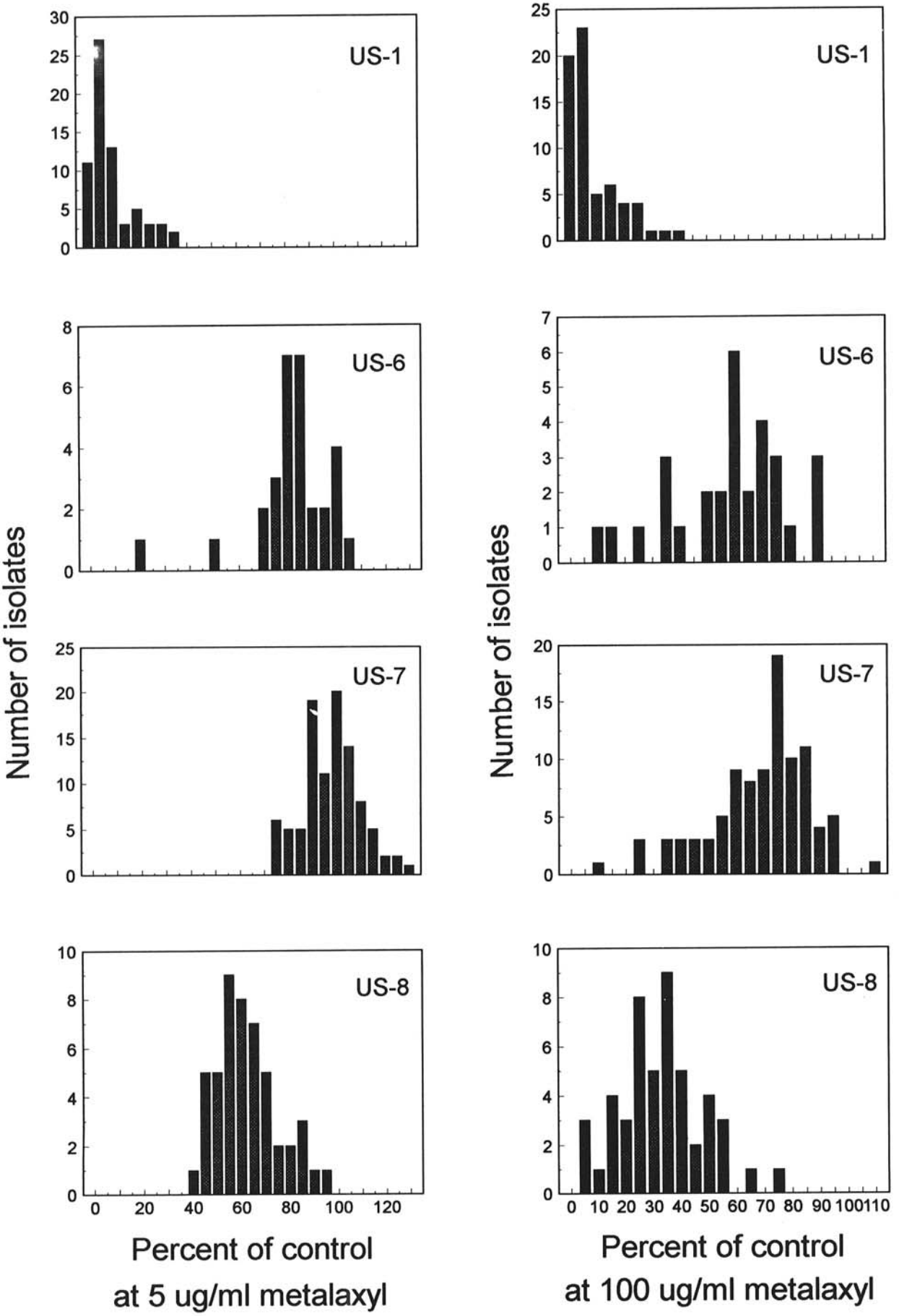

Fig. 1. Response of *Phytophthora infestans* isolates to metalaxyl (percent growth at 5 and 100 µg of metalaxyl per ml relative to the dimethylsulfoxide-only control) for the four most common clonal genotypes (US-1, US-6, US-7, and US-8) in the United States and western Canada during 1992 and 1993. The mean response of each genotype is shown in Table 2.

An interesting pattern emerged when metalaxyl resistance was analyzed according to clonal genotype (Table 2; Fig. 1). The pattern of resistance for most genotypes was unimodal (Fig. 1). All isolates of each lineage were either resistant or sensitive, with the exception of US-6. Although most US-6 isolates were resistant, one was sensitive (Fig. 1). The sensitive isolate was collected from tomatoes grown in California during 1989 (21), whereas all other US-6 isolates were collected during 1992 and 1993. The mean levels of resistance among all four common genotypes were significantly different from each other (t tests, $P < 0.05$). Among 45 US-7 isolates from fields with known spraying histories, isolates from unsprayed fields ($n = 20$) were actually slightly more resistant (mean growth on 5 µg of metalaxyl per ml was 102.8% of the control) than those from sprayed fields ($n = 25$, mean growth 94.7% of the control) ($t = 2.33$, $P < 0.05$). Similar results were obtained with the other resistant genotypes, but the number of isolates from fields that we were sure were not sprayed with metalaxyl was too small for formal hypothesis testing. All of the resistant lineages were those identified as probable recent immigrants (21,27). Isolates of other genotypes (US-1 and BC-1) were either sensitive or had a low level of resistance. Thus, with the exception of one US-6 isolate, metalaxyl resistance was absolutely correlated with clonal genotype.

The US-6 and US-7 genotypes grew almost as rapidly at 100 µg of metalaxyl per ml as at 5 µg (Fig. 1). However, growth of US-8 isolates was much less at 100 µg than at 5 µg of metalaxyl per ml (Fig. 1). These isolates showed a response to metalaxyl that was intermediate between the extreme sensitivity of US-1 isolates and the high level of resistance in US-6 and US-7 isolates (Table 2; Fig. 1). For comparison, analysis of 29 US-1 isolates collected from a single field in New York during 1987 shows the extreme level of sensitivity in populations prior to the most recent migrations (Fig. 2).

Analyses of European isolates collected during the early 1980s revealed resistance both in old and recently immigrated clonal lineages. Four isolates had the US-1 clonal genotype (22). Two of these were from northern Wales and were sensitive; the other two were from Ireland and were resistant, including isolate K1067, one of the first resistant isolates from Ireland in 1980 (3). This isolate had the US-1 genotype and grew at 63% of the control on 5 µg of metalaxyl per ml. Among 23 isolates from the Netherlands collected during 1980 to 1985, all appeared to be members of recently immigrated clonal lineages (22); 9 of these were sensitive to metalaxyl, and 14 were resistant. Among seven isolates collected in Israel from 1984 through 1986, four were resistant, and three were sensitive. However, on the basis of mating type, allozyme, and DNA fingerprint analyses, all seven isolates comprised a single clonal lineage (22).

Field tests confirmed that our criterion for resistance in the in vitro experiments (growth on 5 µg of metalaxyl per ml greater than 40% of the control) also corresponded to resistance in vivo. Epidemics progressed rapidly in both experiments. The time between inoculation to 90% disease was about 24 days in 1993 and 25 days in 1994. In 1993 against the US-6 isolate, metalaxyl alone had no effect on epidemic development (Fig. 3A). Metalaxyl alone also had no significant effect on epidemic development by the US-8 isolate in 1994 (Fig. 3B). However, increasing doses of metalaxyl did appear to reduce epidemic development slightly (data not shown).

The effects of metalaxyl + mancozeb were investigated for both US-6 and US-8. In 1994 against the US-8 isolate, there were significant dosage responses (slope $\neq 0$, $P < 0.05$) for both mancozeb and mancozeb + metalaxyl. However, the dose-response regressions for these two treatments were not significantly different (data not presented). There was no significant dose response for metalaxyl alone. Variances were too high to detect significant dose responses for the US-6 isolate in 1993. However, at the highest dose of mancozeb + metalaxyl, there was significantly less disease ($P < 0.05$) than at the highest dose of mancozeb alone (Fig. 3A).

DISCUSSION

Resistance of *P. infestans* to metalaxyl in the United States and Canada only occurred in genotypes that appeared to be recently introduced. For example, US-7 and US-8 were probably introduced during or shortly before 1992 (27). The probable source population for these recent migrations is in northwestern Mexico (26), where the frequency of resistance was near 100% as of 1989 (32). Therefore, it seems highly likely that resistance of *P. infestans* to metalaxyl in the United States and Canada originated by migration. Two predictions based on the migration hypothesis are that clonal genotypes should be uniformly resistant (have a unimodal distribution), and the level of resistance should be unrelated to the fungicide history of a field. The data fit both predictions for the US-7 and US-8 genotypes. All US-7 and US-8 isolates were resistant, including those that came from unsprayed fields. For example, many US-7 isolates were obtained from tomatoes in home gardens that were never sprayed with metalaxyl, yet these isolates were just as resistant as those from commercial fields in areas that had been sprayed. Because migration events are probably limited, genetic bottlenecks would severely reduce the amount of variability in new populations. Therefore, even if sensitive isolates were present at a low frequency in the original source populations, they almost certainly would have been eliminated by genetic drift during the founding by migration of new populations in the United States and Canada.

The probable origin of resistance in the US-6 genotype is not as clear. One US-6 isolate from California collected during 1989 was sensitive, whereas all US-6 isolates collected during 1992 and 1993 were resistant. US-6 was probably first introduced into California from northwestern Mexico during the late 1970s or early 1980s (21), and was definitely in California by 1982 (21). Therefore, the first migration of US-6 occurred prior to the widespread use of metalaxyl and most likely contained only sensitive isolates. Thus, one possibility is that only sensitive US-6 isolates were introduced initially, and resistant mutants were selected out of the sensitive population after migration.

Another possibility is that there was a second migration of US-6 from northwestern Mexico during the late 1980s or early 1990s. Severe late blight epidemics occurred in northwestern Washington beginning in 1990 (13) after a hiatus of several years (20). Isolates collected during the 1990 epidemic were all classified as inter-

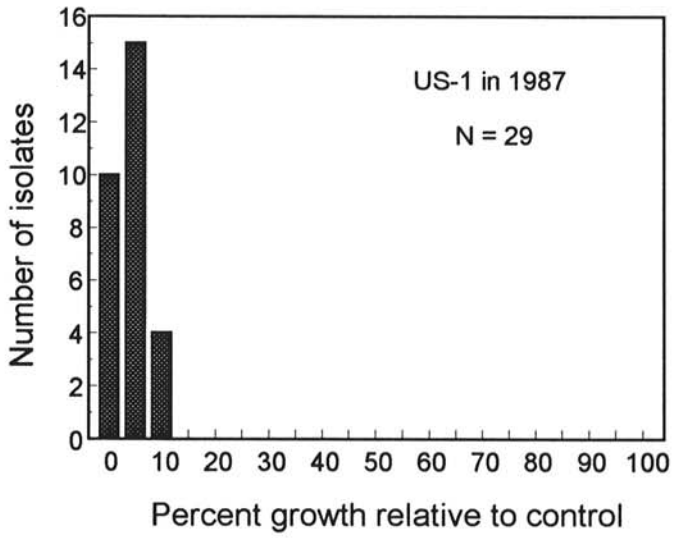

Fig. 2. Response of 29 isolates of *Phytophthora infestans* (US-1 genotype) recovered during 1987 from a single field in Montgomery County, NY, to metalaxyl (expressed as percent growth at 5 µg of metalaxyl per ml relative to the dimethylsulfoxide-only control). This shows the extreme sensitivity of a typical field population prior to the most recent migrations.

mediate or resistant (13). All of these isolates grew at more than 40% of the control on 10 μg of metalaxyl per ml (13) and would be considered resistant based on our criterion. Subsequent analyses revealed that these isolates had the US-6 genotype (21). If resistance was being selected from an initially sensitive population during the 1990 epidemic, we would expect a bimodal distribution containing both sensitive and resistant individuals (13,19). Instead, all US-6 isolates collected in the United States and Canada since 1990 have been resistant. US-6 in northwestern Mexico (26) was nearly 100% resistant by 1989 (32). Because other genotypes (e.g., US-7 and US-8) appear to have been introduced from northwestern Mexico during the early 1990s (27), it seems highly likely that the resistant US-6 isolates from the 1990 Washington epidemics also could have been recent immigrants. Analyses of additional US-6 isolates from the late 1980s are required to test this hypothesis rigorously.

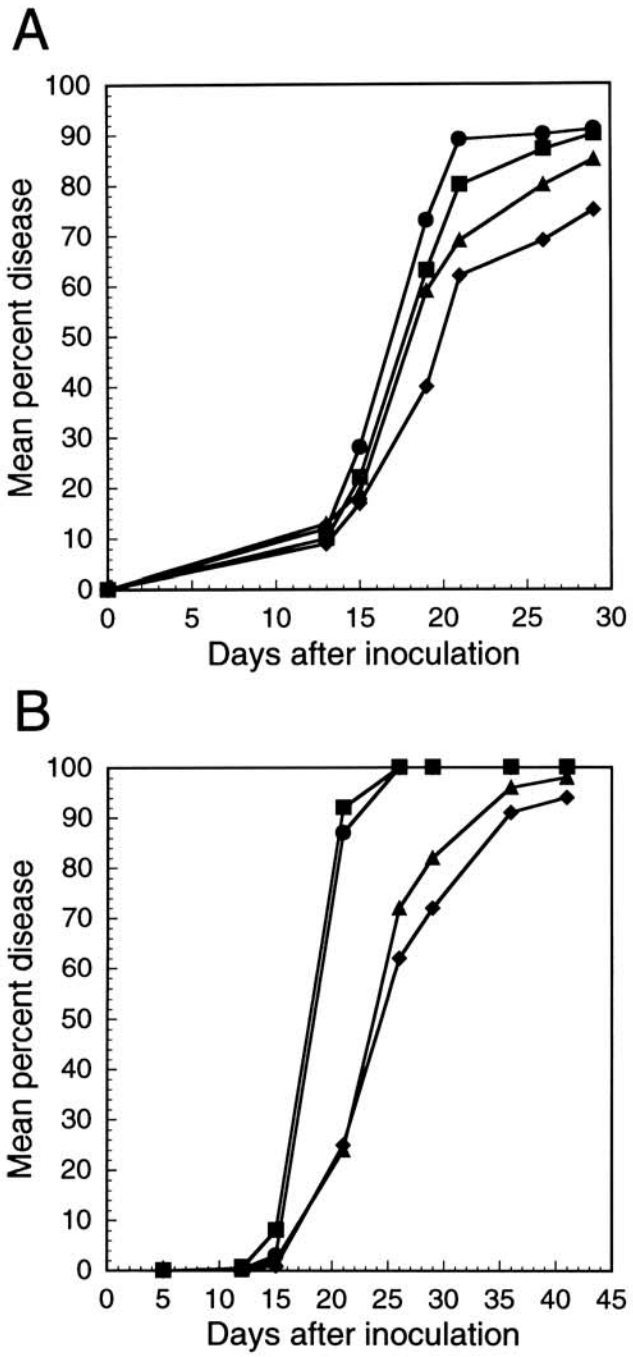

Fig. 3. Disease progress curves at the highest rates of fungicide application (rates are given in Table 3). **A,** The 1993 epidemic with the US-6 genotype. **B,** The 1994 epidemic with the US-8 genotype. Fungicides: ■ = control (no fungicide); ● = Ridomil 2E (metalaxyl alone); ▲ = Dithane M-45 (mancozeb alone); and ◆ = Ridomil MZ58 (metalaxyl + mancozeb).

The pattern for US-1 isolates was distinctly different from that for US-6, US-7, and US-8. All US-1 isolates were sensitive; most did not grow at all on metalaxyl-containing media. The sample of US-1 isolates from a single field in New York in 1987 (Fig. 2) typifies the extreme sensitivity of most populations with this genotype. The slight variation among US-1 isolates collected during 1992 and 1993 may be the result of selection for minor genes within the United States and Canada. Although no unambiguously resistant US-1 isolates were detected in the United States or Canada, they have been detected in the Philippines (30), in addition to Europe, so it seems likely that they will eventually occur in Canada and the United States. Careful management of metalaxyl use may have minimized selection for resistant mutants within the US-1 clonal lineage in northern North America.

In contrast, resistance within US-1 must have developed rapidly in Europe, because one of the first resistant isolates (K1067) from Ireland in 1980 (3) had the US-1 genotype. More intensive use of metalaxyl and larger population sizes of *P. infestans* may have increased the response to selection in Europe compared to the United States and Canada. There was polymorphism for resistance in the single "new" clonal lineage identified among a limited sample from Israel and among new genotypes in the Netherlands. Thus, in addition to the old US-1 clonal lineage, resistance also appears to have arisen in some of the new genotypes in Europe, as has been noted by other investigators (10,16). Because all of the known migrations of *P. infestans* from Mexico into Europe (22,38) occurred before the use of metalaxyl, resistance in Europe probably arose by in situ selection after migration.

Field experiments confirmed the utility of the in vitro assays. In two independent tests (US-6 in 1993 and US-8 in 1994), isolates with typical resistance scores for their genotypes on the agar assay also proved resistant under field conditions. This was not surprising, because there were disease-control failures when metalaxyl was used against populations consisting of US-6, US-7, and US-8 genotypes but not US-1. Metalaxyl has been repeatedly demonstrated to be effective against sensitive isolates (2,18) and can even suppress established epidemics (18). Therefore, even though it was not possible to compare resistant and sensitive isolates in the same field experiment, it seems quite certain that the inability of metalaxyl to control disease was due to resistance. Metalaxyl alone had no effect on epidemics caused by the US-6 and US-8 genotypes, whereas both genotypes were affected by mancozeb. Interestingly, metalaxyl + mancozeb gave slightly better control than either compound alone, even though metalaxyl by itself had no effect. This may indicate synergy (a greater than additive effect) between the two compounds (35). However, the synergistic effect, if present, is small and needs to be confirmed by additional experimentation.

Resistance to metalaxyl in *P. infestans* appeared to be inherited as a single incompletely dominant gene in the only published genetic analysis (37). This conclusion is somewhat tentative because progeny from some backcrosses did not segregate as expected according to the single-gene model (37). However, the hypothesis of a single incompletely dominant gene has been supported in subsequent genetic analyses of *P. sojae* (1) and the related Oomycete *Bremia lactucae* (8,9), of *P. capsici* somatic hybrids (31), and of *P. erythroseptica* field isolates (24). Metalaxyl resistance segregated as a single dominant gene in genetic analyses of laboratory-generated mutants of *P. parasitica* (5). However, because the *P. parasitica* isolates in Chang and Ko's study (5) were classified only as resistant or sensitive, it may not have been possible to determine whether dominance was complete or incomplete.

Isolates tested in the current study were easily classified as resistant or sensitive, as expected if resistance is under single-gene control. Variation within genotypes could be due to the action of modifier genes but is more likely due to experimental error. These

isolates were tested over a 3-year period by three individuals using two media (V8 and rye B agars). Although the qualitative results were always the same, small numerical variations in fungicide-resistance scores may have occurred due to differences in growth on different media or measurement biases by individual investigators.

Assuming single-gene inheritance, we can speculate that US-6 and US-7 (growth on 5 µg of metalaxyl per ml typically 80 to 100% of the control) are most likely homozygous for the resistance allele. US-8 and some of the less common genotypes may be heterozygous. US-8 has the *Gpi 100/111/122* genotype and, therefore, is trisomic, at least for the chromosome containing the *Gpi* locus (26). US-8 isolates grew, on average, at 62% of the control on 5 µg of metalaxyl per ml, compared to 80 to 100%, on average, for US-6 and US-7 isolates. One intriguing possibility is that US-8 isolates also are trisomic and heterozygous for the chromosome containing the metalaxyl-resistance locus with the heterozygous genotype S/R/R. If homozygous resistant diploids (R/R) grow at near 100% and sensitive homozygotes (S/S) at near 0% of the control and if resistance is incompletely dominant, then normal heterozygotes (S/R) might be expected to grow near 50% of the control. Trisomic S/R/R heterozygotes might grow at 67% of the rate of the homozygous (R/R) isolates on metalaxyl or around the 62% seen for US-8. Obviously, this hypothesis needs to be tested by thorough genetic analyses.

Metalaxyl resistance is now widely distributed in the United States and western Canada, along with both mating types. Because resistance was most likely introduced in immigrant genotypes from northwestern Mexico, there is probably nothing that could have been done to prevent it by growers in the United States or Canada. Furthermore, resistance gave the immigrant genotypes a large selective advantage and, thus, probably has played a role in the rapid displacement of US-1 by the new genotypes. Even so, US-1 isolates are still common in some locations and are effectively controlled with metalaxyl, so it is imperative for growers to know which genotypes are present in seed potatoes and in their fields. Although it is currently easy to monitor the spread of different genotypes by a rapid *Gpi* assay (25), this will change as soon as sexual reproduction becomes common. Sexual reproduction has already occurred in British Columbia (27) and will probably generate a population that is highly heterogeneous for metalaxyl sensitivity.

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