Sensitivity of *Phytophthora infestans* Populations to Metalaxyl in Mexico: Distribution and Dynamics

J. M. MATUSZAK, Department of Plant Pathology, Cornell University, Ithaca, NY 14853; J. FERNANDEZ-ELQUEZABAL, National Potato Program, INIFAP, Toluca, Mex., Mexico; W. K. GU, Department of Plant Pathology, Cornell University, Ithaca, NY 14853; M. VILLARREAL-GONZALEZ, National Potato Program, INIFAP, Toluca, Mex., Mexico; and W. E. FRY, Department of Plant Pathology, Cornell University, Ithaca, NY 14853

ABSTRACT

Matuszak, J. M., Fernandez-Elquezabal, J., Gu, W. K., Villarreal-Gonzalez, M., and Fry, W. E. 1994. Sensitivity of *Phytophthora infestans* populations to metalaxyl in Mexico: Distribution and dynamics. Plant Dis. 78:911-916.

Sensitivity to metalaxyl was determined for 966 isolates of *Phytophthora infestans* from seven locations in the states of Chiapas, Coahuila, Mexico, Michoacan, Puebla/Veracruz, and Sinaloa in Mexico. Isolates were collected from 1983 through 1989 in Mexico State and in 1988 or 1989 elsewhere. Sensitivity to metalaxyl was determined in vitro as radial growth on metalaxyl-amended medium, after comparison with the floating-leaf-disk method indicated the techniques provided equivalent results. Metalaxyl-sensitive isolates predominated in collections from Chiapas and Michoacan in 1989 and from Mexico State in 1983, 1984, and 1986. Sensitivity varied within some collections, and the frequency of sensitive isolates varied among collections. The overall frequencies of sensitive isolates from wild *Solanum* species in the large 1988 collection or from tomatoes were not significantly different from the frequency of sensitive isolates from nearby potatoes. In Mexico State, a major potato production area, the frequency of metalaxyl-sensitive isolates was higher in 1983, 1984, and 1986 than in 1987, 1988, and 1989. The frequency of sensitive isolates remained stable during epidemics on untreated potatoes repeatedly sampled in 1988 in the Toluca Valley of western Mexico State. No change occurred in the frequency of sensitive isolates collected from the same untreated site from 1988 to 1989.

Additional keywords: fitness costs, fungicide resistance

RESUMEN

La sensibilidad al metalaxyl fué determinada para 966 aislamientos de *Phytophthora infestans* de siete localidades in los estados de Chiapas, Coahuila, México, Michoacán, Puebla/Veracruz, y Sinaloa en México. Los aislamentos fueron collectados de 1983 a 1989 en el Estado de México y en 1988 o 1989 en otras partes. La sensibilidad al metalaxyl fué determinada en vitro como un crecimiento radil sobre un medio de cultivo reformado con metalaxyl, después de la comparación con el método de disco-hoja-flotante. Los resultados indicaron que las técnicas usadas fueron equivalentes. Los aislamientos con sensibilidad al metalaxyl predominaron en las colecciones de Chiapas y Michoacán en 1989 y las del Estado de México en 1983, 1984, y 1986. Se encontró variación en la sensibilidad dentro de algunas colecciones y la frecuencia de aislameintos sensitivos varió entre las colecciones. Las frequencia de aislamiento sensibiles de especies silvestres de *Solanum*, en la gran coleccion de 1988, o de tomates no fueron significativamente diferentes de la frecuencia de aislamiento sensitivos de papas. En el Estado de México en el área principal de producción de papas la frequencia de los aislamientos sensibles al metalaxyl fué más alta en 1983, 1984, y 1986 que en 1987, 1988, y 1989, la frequencia de los aislamientos sensibles permaneció estable durante las epidemias sobre papas sin tratamiento que se muestrearon repetidamente durante 1988 en el Valle de Toluca al oeste del Estado de México. Ningún cambio ocurrio en la frequencia de los aislamientos sensibles colectados de el mismo sitio sin tratamiento de 1988 a 1989.

Palabras clave adicionales: costo de resistencia a fungicidas, resistencia a fungicidas

The work described in this paper is part of an effort to understand the population genetics and global diversity of *Phytophthora infestans* (Mont.) de Bary (12–15,36,37), including a major effort in Mexico (17,18,25,39,41). Populations in Mexico are important because Mexico is believed to be the center of origin (29,30) and is known to be the area of greatest genetic diversity for biochemical and molecular markers (12,13,15,17,25, 32,36,39) and for R-gene virulence factors (31,40). Mexico is also the area in which the A2 mating type was discovered (16,29) and is dominated by isolates with diploid genomes (18,33,41). In contrast, populations from other countries are primarily polyploid (33,38, 41–43,45).

Accepted for publication 2 July 1994.

While collecting *P. infestans* in the Toluca Valley of Mexico State during 1988, we were alerted by a grower to a disease control failure in a metalaxyl-treated field. Resistance to the systemic phenylamide fungicide metalaxyl in *P. infestans* had been documented in Europe (1,6,10,19) and the Middle East (3). The extent of resistance of *P. infestans* to metalaxyl in Mexico was unknown.

We decided to evaluate Mexican isolates of *P. infestans* for sensitivity to metalaxyl. This evaluation could provide a baseline of information on the sensitivity of *P. infestans* to metalaxyl in populations with high genetic diversity where sexual reproduction is common. As genetic diversity and the potential for sexual reproduction increase worldwide (13), the information may help develop effective integrated management strategies in other locations.

Objectives of this study were to: 1) determine the geographic extent of metalaxyl sensitivity of *P. infestans* populations in Mexico, 2) detect any chronological changes in the frequency of sensitive isolates from central Mexico during the 1980s, and 3) determine if the frequency of sensitive individuals changed during an epidemic within a single season in fields not treated with metalaxyl.

MATERIALS AND METHODS

Collections of isolates. Isolates used in this study (Table 1) were obtained either from a worldwide collection maintained at Cornell University (isolates of 1983–1987) or from the field (isolates of 1988 and 1989). Isolates from the collection had been stored either under mineral oil at 18 C or in 15% dimethyl sulfoxide (DMSO) in distilled water at −135 C. Isolates from storage were plated initially onto rye B agar (2) amended with β-sitosterol (50 mg/L) and then transferred to rye A agar (2) in preparation for analysis. Isolates of 1988 and 1989 were added to the collection.

Isolation was done according to standard procedures (17). Each isolate was from a discrete lesion on a different plant on a single collection date. Previous analyses had indicated that single lesions from natural inoculations rarely contain a mixture of isolates (S. B. Goodwin, *personal communication*). The edge of a lesion was excised and placed in 95% ethanol for 10–15 sec, placed in 0.5% aqueous sodium hypochlorite for 3–5 min, rinsed with sterile water, and placed on V8 agar (28) amended with antibacterial compounds (20 mg/L rifamycin, 50 mg/L polymyxin B sulfate, and 200 mg/L ampicillin) and fungicides (67 mg/L PCNB 75WP and 100 mg/L benomyl

50WP). Isolates were transferred to, and maintained on, rye A agar amended with the same antibacterial compounds and then were stored under oil and cryogenically as described above.

Sources of collections. Isolates were collected from commercial fields, cultivar trials, volunteer potatoes in maize fields that had been in potato production the previous year (1987), and wild *Solanum* species found growing along drainage and irrigation ditches and in waste areas. Fungicides were not used in the year of collection on the cultivar trials, volunteer plants, or wild *Solanum* species. Mancozeb was used on all commercial fields, and metalaxyl was used on some. The three sites repeatedly sampled in 1988 were not sprayed with fungicide in 1988. The site of the 1989 collection in the state of Mexico was not sprayed with fungicides in 1989.

Isolates from 1983–1987 were from the state of Mexico. The majority (20 of 32) of the 1983 isolates came from the international late blight trials of the Mexican National Potato Program in Banco-Metapec (Fig. 1, site 16) and included both potatoes and wild *Solanum* species. The other 12 isolates included six from nearby fields in the valley and six from commercial fields on the slopes of the Nevado de Toluca, the volcano southwest of Toluca. Four of the six isolates from Chapingo, in the eastern part of Mexico State (Fig. 2), collected in 1984 were from a potato research plot. The other two, isolated from *S. cardiophyllum* Lindl., were provided by J. Galindo. The isolates of 1986 included four from research plots of J. Parker in fields adjacent to the Banco and two isolates from A. Rivera-Peña collected on the Nevado de Toluca. The 1987 and 1989 Toluca collections were all from the Banco.

Collections from the Toluca region in 1988 were made from 31 sites in 20 locations (Fig. 1) at elevations of 2,500–3,300 m. Sites within 2 km of one another are identified as single locations. Sites were mostly fields but also included areas where wild *Solanum* species were found. Most isolates were taken from the cultivar Alpha, the predominant cultivar grown commercially. Many commercial fields had been sprayed regularly with mancozeb on a 5- to 7-day schedule, and a few had been sprayed with metalaxyl. Therefore, late blight was not abundant.

Three sites in 1988 were sampled regularly as epidemics progressed. These sites—Atizapan, Banco-Metapec, and Calimaya (Fig. 1)—are located in the Toluca Valley within 10 km of each other. No fungicides had been used on the sampled plots, and only mancozeb had been used on adjacent or nearby plots. At Atizapan and Banco, where potatoes were grown in plots, isolates all represented different rows. At Calimaya, potatoes were growing as volunteers in a maize field and sampling was done on transects.

Additional collections were made in northern Mexico in 1988 and 1989 and in central and southern Mexico in 1989 (Fig. 2). Collections made near Saltillo in Coahuila State in 1988 were from two different commercial potato fields. The fields were about 15 km apart and had been sprayed with both metalaxyl and mancozeb. Collections made near Los Mochis in Sinaloa State in 1989 were from 21 commercial fields (five potato and 16 tomato fields) described by Goodwin et al (17). These fields had been sprayed with both metalaxyl and mancozeb. Isolates from east-central Mexico were from five commercial fields near and between Perote, Veracruz State, and Ciudad Serdan, Puebla State. Information about fungicide use on these fields was unavailable. The west-central Mexican isolates were collected near the town of Patzcuaro, Michoacan State, from unsprayed, naturally inoculated plants within a fungicide (including metalaxyl and mancozeb) demonstration plot. The southern Mexican isolates were from three commercial fields near San Cristobal, Chiapas State. These fields had been sprayed with mancozeb and metalaxyl.

Table 1. Collection data on isolates used in this study

State City	Date collected	No. of isolates	Collectors
Chiapas	Sept. 1989	10	J. M. Matuszak, A. Castro-Gallardo
Coahuila	Aug. 1988	40	W. E. Fry
Mexico			
Chapingo	1984	6	P. W. Tooley, J. Galindo
Toluca	Aug. 1983	32	P. W. Tooley, A. Rivera-Peña
	July 1986	6	J. Parker, A. Rivera-Peña
	July 1987	12	J. Parker, A. Rivera-Peña
	July–Aug. 1988	703	J. M. Matuszak, J. Fernandez-Elquezabal, J. Gomez
	Aug.–Sept. 1989	58	J. M. Matuszak
Michoacan	Sept. 1989	9	J. M. Matuszak, D. Isauro-Jeronimo, V. Cerdeneta, M. Campo-Guttierez
Puebla/Veracruz	Sept. 1989	38	J. M. Matuszak, A. Paredes, A. Ranhel
Sinaloa	Mar. 1989	52	S. N. Bergeron

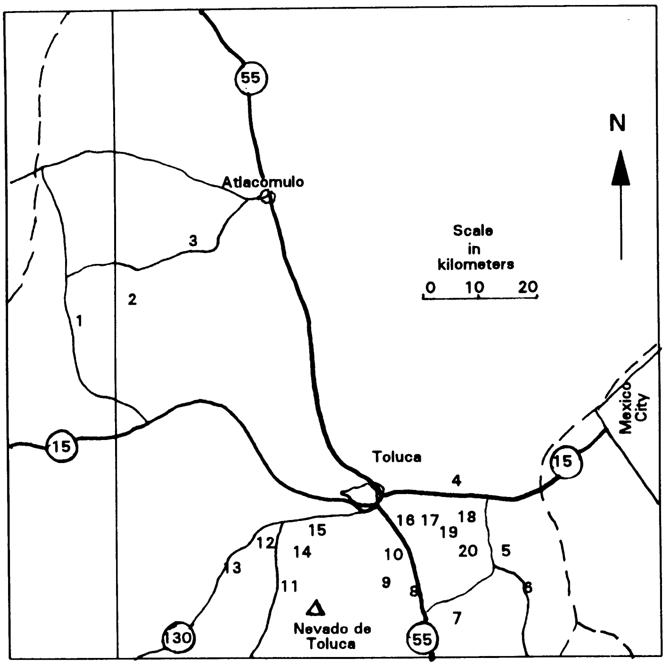

Fig. 1. Collection sites in the Toluca area of western Mexico State, 1988. Dashed lines represent state boundaries. The top edge of the figure represents 20° north latitude, and the vertical line near the left of the figure represents 100° west longitude. Solid lines represent roads; route numbers are circled. Collection sites are indicated by numbers and are named for a nearby village, town, or landmark: 1 = Guadalupe Buena Vista, 2 = San Jeronima de las Dolores, 3 = San Felipe de Progresso, 4 = Lerma, 5 = Santiago Tianguistenco, 6 = San Nicolas Coatepec, 7 = Tenango de Artista, 8 = San Rayon, 9 = Calimaya, 10 = Ocatlan, 11 = Raices, 12 = La Puerta, 13 = Meson Viejo, 14 = Oja de Agua, 15 = San Juan Huerta, 16 = Banco-Metapec (INIFAP Programa de Papas), 17 = near Conalep, east of Metapec, 18 = west of Atizapan, 19 = Atizapan-CIMMYT, and 20 = Chapultepec; sites 9, 16, and 19 were sampled repeatedly.

Metalaxyl sensitivity. We compared the two methods commonly used to assess metalaxyl sensitivity: measurement of radial growth on metalaxyl-amended agar medium (5,34,35) and visual assessment of sporulation on potato leaf disks floating on metalaxyl-containing water (6,35). The same 20 isolates were tested with both methods.

For the leaf-disk assay, disks 2 cm in diameter were cut with a cork borer from fully expanded leaflets of potato cv. Norchip, grown in a greenhouse. Disks were floated abaxial side up on solutions of 0, 5, and 100 μg/ml of metalaxyl (5 ml), one disk per 5-cm petri plate. Technical-grade (90%) solid metalaxyl (Ciba Plant Protection, Greensboro, NC) was first dissolved in DMSO. The final fungicide concentrations were 5 and 100 μg/ml, and DMSO concentration was 0.1%. Disks floated on distilled water containing DMSO without metalaxyl served as controls. Each disk was inoculated with 30 μl of a suspension containing 1–2 $\times 10^4$ sporangia per milliliter applied with a micropipette. The closed plates were kept at 18 C with 14 hr light per day. Treatments were replicated six times and evaluations combined. After 7 days the proportion of total disk surface with sporulation was assessed visually as described by Davidse et al (6). Replicated assessments were averaged and expressed relative to sporulation in the absence of metalaxyl.

For the amended agar assay, 9-mm agar disks with mycelium were taken from the margins of actively expanding cultures growing on Rye A medium in petri plates. All plugs for a test came from the same colony. The plugs were transferred to clarified V8 or rye B plates amended with metalaxyl in DMSO to a final concentration of 5 or 100 μg/ml. The control was amended with DMSO without metalaxyl. There were two replications, and two perpendicular measurements of diameter were made for each replication; results for a given treatment for a given isolate were averaged. Evaluations were generally made after 7–14 days of incubation (18 C, 14 hr of light) when the control colonies (0 metalaxyl) were at least 3 cm in diameter but had not reached the margin of the plate. Radial growth as a percentage of control (growth without metalaxyl) was determined for each isolate at each concentration. Some slower growing isolates were evaluated after 21 days. Most evaluations were conducted on V8 agar. Some isolates grew very slowly on this medium, and these isolates were tested on a clarified rye B medium. Standards consisting of an isolate known to be highly sensitive and an isolate known to be highly insensitive to metalaxyl were included in each trial. After comparison of the two methods gave similar results, the entire collection was evaluated with the radial growth assay.

Storage under oil or at −135 C and maintenance on rye A did not affect resistance. Four isolates that were used as standards for resistance gave consistent results over a 3-yr period whether the culture came from oil, cryogenic storage, or subculture on rye A.

Summary statistics were calculated for comparison purposes. Although not all populations appeared normally distributed, the mean relative growth rate at 5 and 100 μg/ml of metalaxyl for a population was calculated from relative growth rates of all isolates representing the population. Standard deviations and standard errors were calculated.

RESULTS

Assay comparison. The agar and floating leaf disk assays gave equivalent results for 20 isolates that were tested by both methods. The relationship between the methods at 5 μg/ml of metalaxyl was described by the line $y = (-15.8) + 1.21x$ ($r^2 = 0.94$), and that at 100 μg/ml was described by the line $y = (-2.07) + 0.997x$ ($r^2 = 0.98$) (Fig. 3).

Incidence of metalaxyl sensitivity in Mexican isolates. The 966 isolates varied greatly in the degree of growth inhibition caused by metalaxyl (Fig. 4). The distribution of sensitivity at 5 μg/ml was bimodal (Fig. 4). One peak consisted of a large number of isolates, completely inhibited, with relative growth rates <20% of their controls, and the other peak consisted of a large number of isolates that were generally unaffected, with growth rates >80% of the control. The bimodal distribution detected at 5 μg/ml of metalaxyl was consistent with the definition by Daggett et al (5) of a sensitive isolate, i.e., one with relative growth rate <40% of its control at both 5 and 100 μg/ml. All isolates that grew <40% of the control at 5 μg/ml also grew <30% of the control at 100 μg/ml, and 90% of the sensitive isolates detected at 5 μg/ml had growth rates <20% of the control at 100 μg/ml.

Geographic distribution of metalaxyl resistance. Both sensitive and insensitive isolates were detected at all but one location in the 1988 collection from Toluca in the state of Mexico (Fig. 1). The one exception was a site near Meson Viejo where no isolates from either potatoes or wild *S. iopetalum* (Bitt.) Hawkes were sensitive. Insensitive isolates were more frequent than sensitive isolates at all locations. Sensitive isolates were identified from two locations (near Tenango de Artista and Santiago Tianguistengo) known to have been sprayed with metalaxyl.

In regions other than the state of Mexico (Fig. 2), there were large differ-

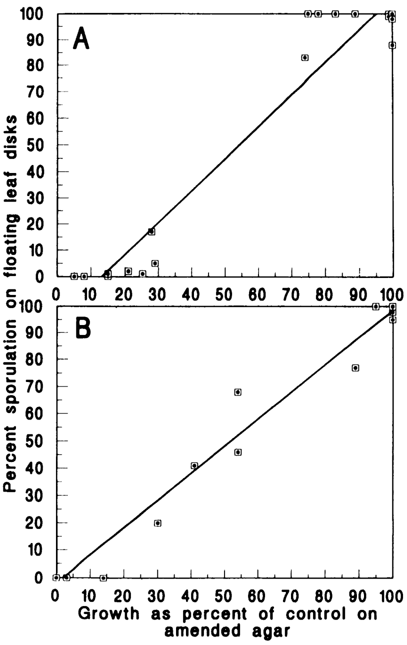

Fig. 3. Comparison of radial growth and floating leaf disk assays for determining metalaxyl sensitivity. Data points are averages of six replicates of 20 isolates. Results of the agar radial growth assay are presented as growth rate relative to control (0 metalaxyl). Results of the floating leaf disk assay are presented as the proportion of leaf area supporting sporulation. (In all cases, 100% of the leaf disk area supported sporulation at 0 metalaxyl.) **(A)** Regression characteristics at 5 μg/ml: r^2 =0.94, slope coefficient = 1.21. **(B)** Regression characteristics at 100 μg/ml: r^2 = 0.98, slope coefficient = 0.997.

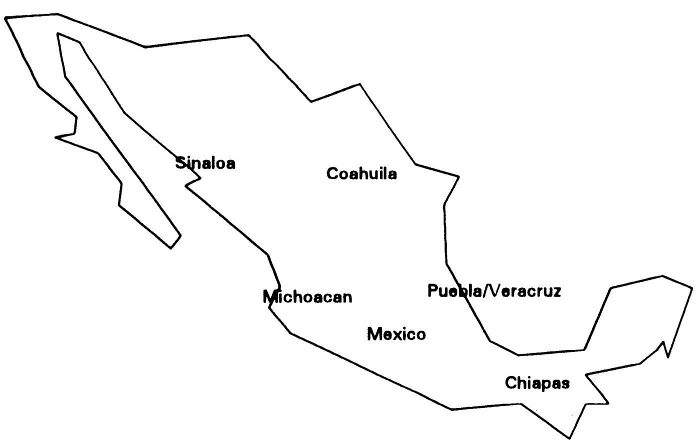

Fig. 2. States of Mexico where collections were made.

ences in frequencies of sensitivity to metalaxyl (Fig. 5). The frequency of sensitivity to metalaxyl was very low in Sinaloa. The isolates from Sinaloa came from 16 tomato and five potato fields, but genetic diversity was low; the population consisted of only a few clones (17) and fungicides were applied regularly. The absence of sensitive individuals and the low genetic diversity were reflected in the relatively restricted range for that population (Fig. 5). All isolates from Chiapas (near San Cristobal de las Casas) were sensitive to metalaxyl. The sample size there was small because of the low level of potato production and the limited occurrence of the disease. Metalaxyl had been used in at least one of the fields from which isolates were collected.

Populations from the states of Coahuila, Michoacan, and Puebla/Veracruz contained isolates with a wide range of sensitivities. The frequency of sensitive isolates from Michoacan was high. Sensitivity was rare in collections from Coahuila and Puebla/Veracruz (Fig. 5). Michoacan has relatively less potato production than Coahuila, an area of rather intense production, or than the Puebla/Veracruz region, which, along with Toluca, has been producing potatoes for decades (44).

The sample from Chapingo (eastern Mexico State) was collected in 1984. In this sample, one isolate (which came from *S. cardiophyllum*) was not sensitive to metalaxyl.

Dynamics of resistance. Samples from the state of Mexico had a dramatic decrease in the frequency of sensitive isolates in the 1980s and an increase in the mean rates of growth of samples at the same time (Fig. 6). In early collections, >80% of the isolates were sensitive, with the mean relative growth rate of isolates at 5 μg/ml <20%, whereas later in the decade, <30% of isolates were sensitive and mean relative growth rates at 5 μg/ml were >50%.

When the same sites were sampled repeatedly in 1988, there were no significant changes in frequency of metalaxyl sensitivity over the sampling period of approximately 40 days (Atizapan, $P > 0.35$; Banco, $P > 0.2$; Calimaya, $P > 0.85$). The percentage of isolates sensitive to metalaxyl remained <50% (Fig. 7). Mean relative growth rates in the presence of metalaxyl (5 μg/ml) ranged from 53 to 73% (SD = ± 24–33%) at Atizapan, from 47 to 63% (SD = ± 27–42%) at the Banco, and from 53 to 58% (SD = ± 33%–37%) at Calimaya.

Role of host. Metalaxyl-resistant isolates occurred with similar frequencies on potatoes, tomatoes, and wild *Solanum* species within the same geographic region. In the large 1988 collection from Toluca, the frequency of metalaxyl sensitivity among isolates from wild hosts was not significantly different from that in the larger population taken from potatoes ($\chi^2 = 0.57$, $P > 0.25$). The only location in which isolates from potatoes and tomatoes occurred together was in Sinaloa, where isolates from either host showed little sensitivity. In the 1983 collection from Toluca, the only two insensitive isolates came from wild *Solanum* species. Similarly, in the 1984 Chapingo collection, the only resistant isolate was from *S. cardiophyllum*. However, the small sample sizes of those collections preclude any general conclusion regarding the relative frequency of resistant isolates on wild vs. cultivated *Solanum* populations. We detected no evidence for an influence of host on the frequencies of metalaxyl sensitivity.

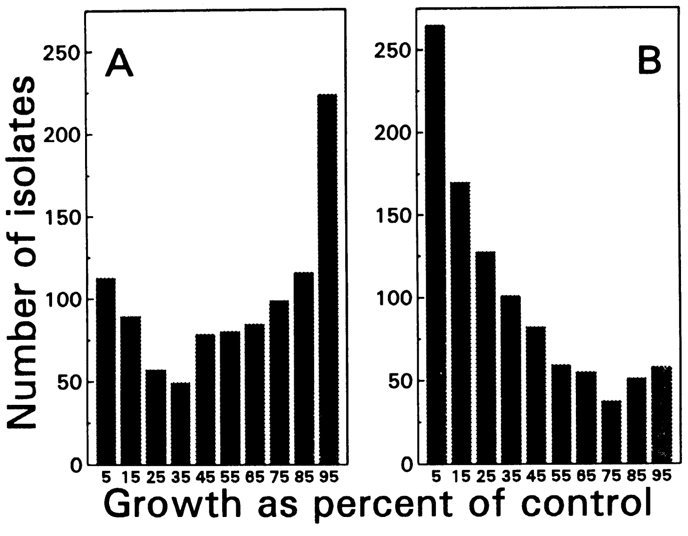

Fig. 4. Relative growth of 966 Mexican isolates of *Phytophthora infestans* on agar with **(A)** 5 μg/ml and **(B)** 100 μg/ml of metalaxyl. Radial growth on agar medium containing metalaxyl was expressed as percentage of growth on agar medium containing 0 metalaxyl. Histogram bars represent 0–9%, 10–19%, 20–29%, 30–39%, 40–49%, 50–59%, 60–69%, 70–79%, 80–89%, and 90–100+% of control values.

DISCUSSION

By the late 1980s, there was a low frequency of metalaxyl-sensitive isolates in most populations of *P. infestans* in the major potato-producing areas of Mexico, but there were substantial differences in the frequencies of sensitive isolates in different regions in Mexico. Collections earlier in the 1980s contained a much higher frequency of sensitive isolates. The frequency of sensitive isolates from wild species of *Solanum* was similar to that obtained from cultivated crops in the large 1988 collection. There was no detectable change in the frequency of sensitive isolates from untreated sites throughout a single-season epidemic in 1988. Neither was there a change in the frequency of sensitive isolates from 1988 to 1989 at the same site (Banco-Metapec).

Examining *P. infestans* within Mexico offers the opportunity to compare the occurrence of metalaxyl resistance in genetically diverse, sexually reproducing, endemic populations with that in asexual, genetically limited, derived populations. In endemic sexually reproducing *P. infestans* populations in the Toluca Valley, variation in sensitivity was high. This diversity may persist for an extended period of time because of long-term survival of oospores in the soil, effectively preventing fixation (100% of alleles in the population) of any character for which selection is not constant and widespread over a long time. Populations with the least diversity, as indicated by standard error of the mean relative growth rate for metalaxyl sensitivity (Chiapas and Sinaloa) (Fig. 5), were those that were strongly clonal (17; J. M. Matuszak, *unpublished*).

Whether resistance to pesticides is the result of preexisting genetic variance (preadaptation) in a population or the result of mutations that occur after exposure to the selection agent has been long debated (4). Crow (4) argued that for insects and insecticides, preadaptation was the norm and insecticides only acted as a selective agent. The fact that resistance in *P. infestans* was found at a low frequency in the 1983 and 1984 collections from wild species supports the possibility that it was preexistent in the population. Detection of metalaxyl-resistant isolates of *P. megasperma* Drechs. in populations apparently unexposed to metalaxyl (20) may also represent a case of preadaptation to metalaxyl in populations of another *Phytophthora* species. Preexisting variants can also be introduced via migration. This may have been the case in Sinaloa, where preexistent insensitive isolates may have been introduced via migration. The migrants may have originated from the endemic population in the central or northeastern Mexican highlands (17,30), which also are areas where Mexican seed potatoes are produced.

We were somewhat surprised to see

no consistent changes in the frequency of sensitive isolates in the absence of selection pressure. If there were small fitness costs, our sample sizes may not have been large enough or over a sufficient amount of time to detect them. If there were large fitness costs associated with resistance, we might have been able to detect an increase in sensitivity. Fitness costs associated with pesticide resistance have been hypothesized since early cases of resistance to chemical pesticides were reported in insect populations (4). Fitness costs are included as a primary factor in explanations of why characters under selection do not always go to fixation.

If fitness costs exist for metalaxyl, it may be possible to prolong the effectiveness of metalaxyl by using the management strategy of periodic product withdrawal to lessen selection and reduce the frequency of resistant isolates. Decreases in the frequency of resistance in the absence of selection pressure are predicted by models that assume a cost to resistance (7,8,26,27). Dowley and O'Sullivan (11) documented a reduction in the frequency of resistant isolates in the Irish population of *P. infestans* after withdrawal of metalaxyl from the market in the early 1980s. Dowley (9) subsequently demonstrated experimentally a fitness cost in the resistant isolates as the basis of the decline. Kadish et al (21,22,24) found an overall higher compound fitness index (based on infection frequency, lesion area, and sporulation

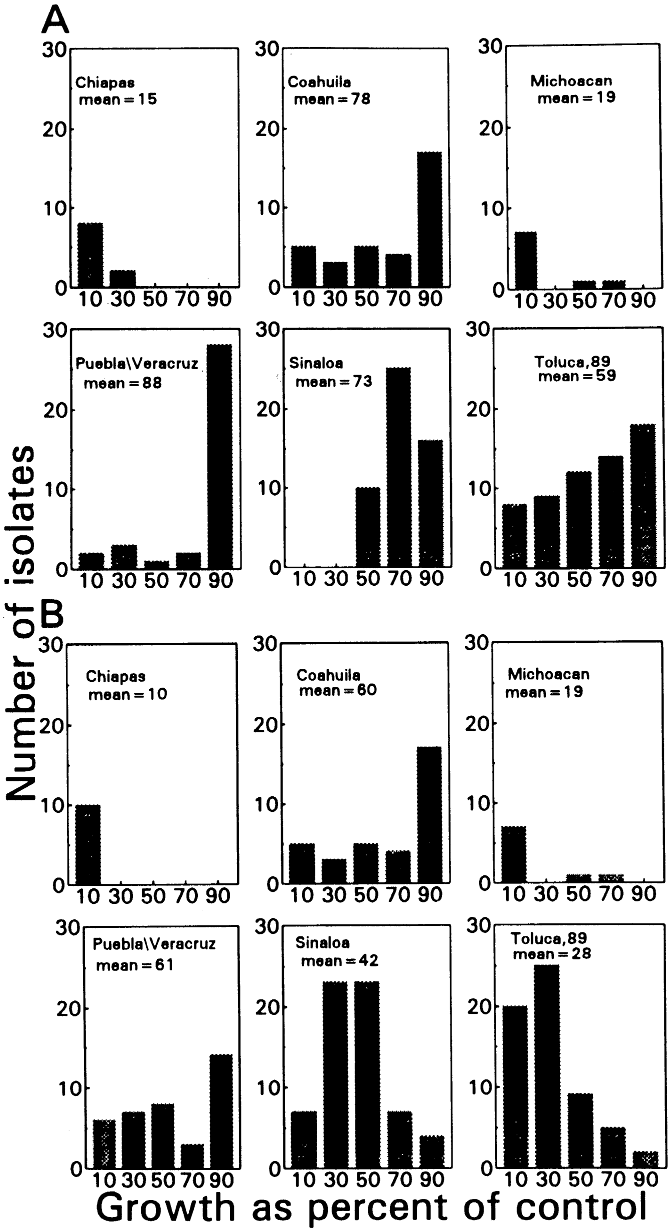

Fig. 5. Sensitivity of *Phytophthora infestans* isolates from different locations in Mexico to **(A)** 5 μg/ml and **(B)** 100 μg/ml of metalaxyl. Sensitivity is represented as radial growth rate relative to that of the control (0 metalaxyl). Histogram bars represent 0–19%, 20–39%, 40–59%, 60–79%, and 80–100+% of control values. Population means were calculated from noncategorized relative growth rates.

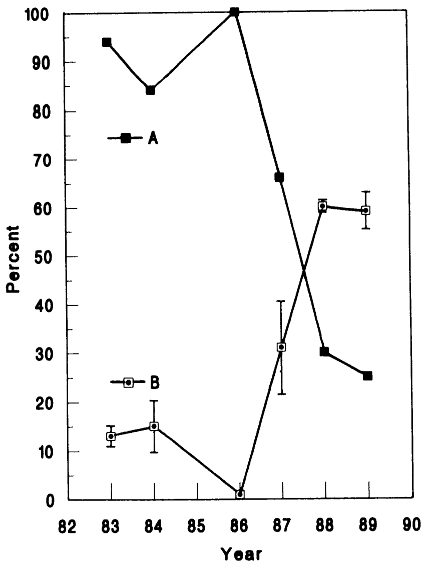

Fig. 6. Frequency of metalaxyl-sensitive isolates and mean growth rates of sample populations of *Phytophthora infestans* in populations from Mexico State during 1983–1989. A = sensitive isolates, i.e., those with growth rates reduced to <40% of the control growth rate (0 metalaxyl) at both 5 μg/ml and 100 μg/ml of metalaxyl; frequency is presented as percentage of the total collection each year. B = mean growth rate of the total collection each year; standard error was calculated for each mean. Thirty-two samples were tested in 1983, 6 in 1984, 6 in 1986, 12 in 1987, 703 in 1988, and 58 in 989. The sample from 1984 came from the Chapingo area of eastern Mexico State; all others came from the Toluca area of western Mexico State.

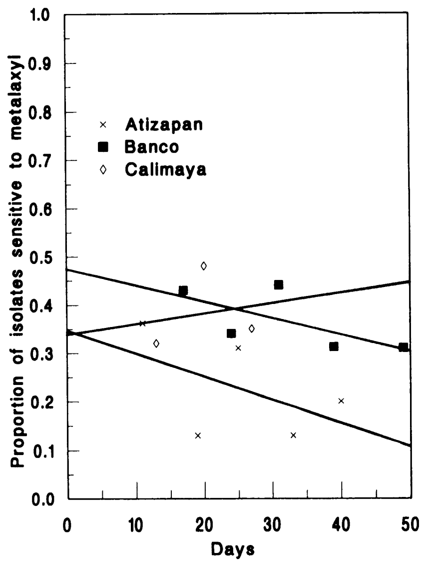

Fig. 7. Frequency of metalaxyl-sensitive isolates of *Phytophthora infestans* over the course of an epidemic in 1988 at three locations in the Toluca Valley in western Mexico State. The sites were not sprayed and were sampled regularly as the epidemic progressed. Regression characteristics: Atizapan, $y = 0.35\text{-}0.0048x$ ($r^2 = 0.26$); Banco, $y = 0.47\text{-}0.0038x$ ($r^2 = 0.45$); Calimaya, $y = 0.33 + 0.0026x$ ($r^2 = 0.04$).

capacity) for metalaxyl-insensitive isolates but later (23) found significantly higher overseason survival for metalaxyl-sensitive isolates. Reduced fitness of insensitive genotypes in a strongly clonal population may be the result of specific associations between the gene(s) conferring insensitivity and the background genotype in which it occurs. It may be that sexual reproduction in central Mexico has broken any association between metalaxyl resistance and reduced fitness. If so, our results illustrate the potential importance of population genetic structure for responses of fungal populations to selective fungicides.

ACKNOWLEDGMENTS

We thank E. Betanzos-Mendoza, J. Amador, and E. Alvarez-Luna for assistance in collecting isolates; M. Ramirez-Gomez and M. Cadena for providing supplies in Mexico in 1988; L. J. Spielman and B. McMaster for assistance in processing isolates in 1988; and W. Sinclair for helpful comments. Supported in part by the Centro Internacional de la Papa (CIP), Cornell University Latin American Studies Program, Cornell Graduate School, USDA CRGO Grant No. 91-37303-4477 and Cornell Hatch Project 153430.

LITERATURE CITED

1. Carter, G. A., Smith, R. M., and Brent, K. J. 1982. Sensitivity to metalaxyl of *Phytophthora infestans* populations in potato crops in south-west England in 1980 and 1981. Ann. Appl. Biol. 100:433-441.
2. Caten, C. E., and Jinks, J. L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Can. J. Bot. 46:329-348.
3. Cohen, Y., and Reuveni, M. 1983. Occurrence of metalaxyl resistant isolates of *Phytophthora infestans* in potato fields in Israel. Phytopathology 73:925-927.
4. Crow, J. F. 1957. Genetics of insect resistance to chemicals. Annu. Rev. Entomol. 2:227-246.
5. Dagget, S. S., Götz, E., and Therrien, C. D. 1993. Phenotypic changes in populations of *Phytophthora infestans* from eastern Germany. Phytopathology 83:319-323.
6. Davidse, L. C., Looijen, D., Turkensteen, L. J., and Van der Wal, D. 1981. Occurrence of metalaxyl-resistant strains of *Phytophthora infestans* in Dutch potato fields. Neth. J. Plant Pathol. 87:65-68.
7. Dekker, J. 1984. The development of resistance to fungicides. Prog. Pestic. Biochem. Toxicol. 4:165-218.
8. Dekker, J. 1986. Preventing and managing fungicide resistance. Pages 347-354 in: Pesticide Resistance: Strategies and Tactics for Management. National Academy Press, Washington, DC.
9. Dowley, L. J. 1987. Factors affecting the survival of metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. Potato Res. 30:473-475.
10. Dowley, L. J., and O'Sullivan, E. 1981. Metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. Potato Res. 24:417-421.
11. Dowley, L. J., and O'Sullivan, E. 1985. Monitoring metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. Potato Res. 28:531-534.
12. Fry, W. E., Drenth, A., Spielman, L. J., Mantel, B. C., Davidse, L. C., and Goodwin, S. B. 1991. Population genetic structure of *Phytophthora infestans* in the Netherlands. Phytopathology 81:1330-1336.
13. Fry, W. E., Goodwin, S. B., Dyer, A. T., Matuszak, J. M., Drenth, A., Tooley, P. W., Sujkowski, L. S., Koh, Y. J., Cohen, B. A., Spielman, L. J., Deahl, K. L., Inglis, D. A., and Sandlan, K. P. 1993. Historical and recent migrations of *Phytophthora infestans*: Chronology, pathways, and implications. Plant Dis. 77:653-661.
14. Fry, W. E., Goodwin, S. B., Matuszak, J. M., Spielman, L. J., Milgroom, M. G., and Drenth, A. 1992. Population genetics and intercontinental migrations of *Phytophthora infestans*. Annu. Rev. Phytopathol. 30:107-129.
15. Fry, W. E., and Spielman, L. J. 1991. Population biology of *Phytophthora infestans*. Adv. Plant Pathol. 7:171-192.
16. Gallegly, M. E., and Galindo, J. 1958. Mating types and oospores of *Phytophthora infestans* in potato fields in the United States and Mexico. Phytopathology 48:274-277.
17. Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N., and Fry, W. E. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82:955-961.
18. Gu, W. K., Spielman, L. J., Matuszak, J. M., Aist, J. M., Bayles, C. J., and Fry, W. E. 1993. Measurement of nuclear DNA contents of Mexican isolates of *Phytophthora infestans*. Mycol. Res. 97:857-860.
19. Holmes, S. J. I., and Channon, A. G. 1984. Studies on metalaxyl-resistant *Phytophthora infestans* in potato crops in south-west Scotland. Plant Pathol. 33:347-354.
20. Hunger, R. M., Hamm, P. B., Horner, C. E., and Hansen, E. M. 1982. Tolerance of *Phytophthora megasperma* isolates to metalaxyl. Plant Dis. 66:645-649.
21. Kadish, D., and Cohen, Y. 1988. Competition between metalaxyl-sensitive and metalaxyl-resistant isolates of *Phytophthora infestans* in the absence of metalaxyl. Plant Pathol. 37:558-564.
22. Kadish, D., and Cohen, Y. 1988. Fitness of *Phytophthora infestans* isolates from metalaxyl-sensitive and resistant populations. Phytopathology 78:912-915.
23. Kadish, D., and Cohen, Y. 1992. Overseasoning of metalaxyl-sensitive and metalaxyl-resistant isolates of *Phytophthora infestans* in potato tubers. Phytopathology 82:887-889.
24. Kadish, D., Grinberger, M., and Cohen, Y. 1990. Fitness of metalaxyl-sensitive and metalaxyl-resistant isolates of *Phytophthora infestans* on susceptible and resistant potato cultivars. Phytopathology 80:200-205.
25. Matuszak, J. M., Goodwin, S. B., Fry, W. E., Villarreal-Gonzalez, M. J., and Fernandez-Elquezabal, J. 1991. The genetic structure of *Phytophthora infestans* populations in the Toluca Valley as determined by molecular markers. (Abstr.) Phytopathology 81:965.
26. Milgroom, M. G., and Fry, W. E. 1988. A simulation analysis of the epidemiological principles for fungicide resistance management in pathogen populations. Phytopathology 78:565-570.
27. Milgroom, M. G., Levin, S. A., and Fry, W. E. 1989. Population genetics theory and fungicide resistance. Pages 340-367 in: Plant Disease Epidemiology. Vol. 2, Genetics, Resistance, and Management. K. J. Leonard and W. E. Fry, eds. McGraw-Hill, New York.
28. Miller, P. M. 1955. V-8 juice as a general purpose medium for fungi and bacteria. Phytopathology 45:461-462.
29. Niederhauser, J. S. 1956. The blight, the blighter, and the blighted. Trans. N.Y. Acad. Sci. 19:55-63.
30. Niederhauser, J. S. 1991. *Phytophthora infestans*: The Mexican connection. Pages 25-45 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, eds. Cambridge University Press, Cambridge, England.
31. Niederhauser, J. S., and Cobb, W. C. 1959. The late blight of potatoes. Sci. Am. 200:100-112.
32. Rivera-Peña, A. 1990. Wild tuber bearing species of *Solanum* and incidence of *Phytophthora infestans* (Mont.) de Bary on the western slopes of the volcano Nevado de Toluca. 3. Physiological races of *Phytophthora infestans*. Potato Res. 33:349-355.
33. Sansome, E., and Brasier, C. M. 1973. Diploidy and chromosomal structure hybridity in *Phytophthora infestans*. Nature 241:344-345.
34. Shattock, R. C. 1988. Studies on the inheritance of resistance to metalaxyl in *Phytophthora infestans*. Plant Pathol. 37:4-11.
35. Sozzi, D., and Staub, T. 1987. Accuracy of methods to monitor sensitivity of *Phytophthora infestans* to phenylamide fungicides. Plant Dis. 71:422-425.
36. Spielman, L. J. 1991. Isozymes and the population genetics of *Phytophthora infestans*. Pages 231-241 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, eds. Cambridge University Press, Cambridge, England.
37. Spielman, L. J., Drenth, A., Davidse, L. C., Sujkowski, L. J., Gu, W. K., Tooley, P. W., and Fry, W. E. 1991. A second world-wide migration and population displacement of *Phytophthora infestans*? Plant Pathol. 40:422-430.
38. Therrien, C. D., Ritch, D. L., Davidse, L. C., Jespers, B. K., and Spielman, L. J. 1989. Nuclear DNA content, mating type and metalaxyl sensitivity of eighty-three isolates of *Phytophthora infestans* from the Netherlands. Mycol. Res. 92:140-146.
39. Tooley, P. W., Fry, W. E., and Villarreal-Gonzalez, M. J. 1985. Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. J. Hered. 76:431-435.
40. Tooley, P. W., Sweigard, J. A., and Fry, W. E. 1986. Fitness and virulence of *Phytophthora infestans* isolates from sexual and asexual populations. Phytopathology 76:1209-1212.
41. Tooley, P. W., and Therrien, C. D. 1987. Cytophotometric determination of the nuclear DNA content of 23 Mexican and 18 non-Mexican isolates of *Phytophthora infestans*. Exp. Mycol. 11:19-26.
42. Tooley, P. W., and Therrien, C. D. 1991. Variation in ploidy in *Phytophthora infestans*. Pages 204-217 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, eds. Cambridge University Press, Cambridge, England.
43. Tooley, P. W., Therrien, C. D., and Ritch, D. L. 1989. Mating type, race composition, nuclear DNA content, and isozyme analysis of Peruvian isolates of *Phytophthora infestans*. Phytopathology 79:478-481.
44. Urgent, D. 1967. The potato in Mexico: Geography and primitive culture. Econ. Bot. 22:108-123.
45. Whittaker, S. L., Shattock, R. C., and Shaw, D. S. 1991. Variation in DNA content of nuclei of *Phytophthora infestans* as measured by a microfluorimetric method using the fluorochrome DAPI. Mycol. Res. 95:602-610.