# IDENTIFICATION OF MATING TYPES AND METALAXYL RESISTANCE IN NORTH AMERICAN POPULATIONS OF *PHYTOPHTHORA INFESTANS*

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**Abstract**

The $A^2$ mating type of *Phytophthora infestans* was first reported in the United States in 1990. Concurrently, *P. infestans* strains resistant to metalaxyl were found in the Pacific Northwest. Collaborative surveys were undertaken during 1991-1993 to investigate the frequency of occurrence of $A^2$ mating types and metalaxyl resistant strains in populations of *P. infestans* isolated from outbreaks of late blight in potato and tomato crops in North America. *In vitro* testing indicated that isolates from the northeastern U.S. and Atlantic Canada were primarily (52/55) metalaxyl sensitive and all were $A^1$ mating types. Among 85 isolates from late blight epidemics in Florida and Texas, greater than 61% were both metalaxyl resistant and $A^2$ mating type. Metalaxyl resistance and $A^2$ mating types were identified also in a few tomato isolates from North Carolina. Although the majority of 134 isolates from the Pacific Northwest (British Columbia and Washington) were metalaxyl resistant, only 2 isolates from Washington were $A^2$ mating types. Among 111 isolates from 2 sites in central Mexico, 63% and 77% were both metalaxyl resistant and $A^2$ mating types. The data indicate also a higher frequency of metalaxyl resistance in $A^2$ isolates, than in $A^1$ isolates, among isolates from Florida and Texas. Highest metalaxyl resistance levels were found, however, in $A^1$ isolates from California, where no $A^2$ isolates were recovered.

**Compendio**

El tipo reproductivo $A^2$ de *Phytophthora infestans* fue reportado por primera vez en los Estados Unidos en 1990. Simultáneamente, se encontraron en el noroeste del Pacífico variantes de *P infestans*, resistentes al metalaxyl. Durante 1991-1993, se llevaron a cabo encuestas colaborativas para investigar la frecuencia conque se presentan los tipos $A^2$ y las variantes resistentes al metalaxyl en las poblaciones de *P. infestans* aisladas, en las epidemias de tizón tardío, de los cultivos de papa y tomate en Norteamérica. La pruebas *in vitro* indicaron que los aislamientos del noreste de los Estados Unidos y de la zona canadiense del Atlántico fueron principalmente (52/55) sensibles al metalaxyl, perteneciendo todos ellos al tipo reproductivo $A^1$. Entre 85 aislamientos de epidemias de tizón tardío en Florida y Texas,

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m as de 61% fueron resistentes al metalaxyl y del tipo reproductivo $A^2$. Se identificaron también la resistencia al metalaxyl y los tipos reproductivos $A^2$ en algunos aislamientos de tomate en Carolina del Norte. Aunque la mayoría de 134 aislamientos del noroeste del Pacífico (Columbia Británica y Washington) fueron resistentes al metalaxyl, sólo dos aislamientos de Washington fueron de los tipos reproductivos $A^2$. Entre 111 aislamientos de dos lugares del centro de México, 63% y 77% fueron tanto resistentes al metalaxyl como de los tipos reproductivos $A^2$. Los datos también señalan una mayor frecuencia de resistencia al metalaxyl en los aislamientos $A^2$ que en los $A^1$ efectuados en Florida y Texas. Sin embargo, se encontraron niveles más altos de resistencia al metalaxyl en los aislamientos $A^1$ de California, donde no se recuperaron aislamientos $A^2$.

## Introduction

Pathogenic strains of *P. infestans* resistant to metalaxyl were first reported in the Pacific Northwest of the United States and Canada in 1991 (7, 8). During extremely favorable weather conditions that had prevailed the previous two years, late blight developed and spread rapidly on metalaxyl-treated potato crops. It was determined that the presence of metalaxyl-resistant strains accounted for the lack of disease control with this systemic fungicide (8). Studies in other countries had similarly established that continued application of metalaxyl to diseased crops had induced a shift in the pathogen population to greater metalaxyl insensitivity, as indicated by the higher frequency of resistant isolates present in late blight leaf samples taken from fungicide-treated fields or from tuber samples obtained from potato storages (4, 9, 22, 27).

Research on the genetic diversity of the late blight pathogen led to postulation that the *P. infestans*: *Solanaceae* pathosystem had central Mexico as its center of origin (13, 14). Recent genetic diversity studies using mating type analyses, virulence loci, and DNA fingerprinting probes have substantiated this hypothesis (15). Until 1984, the $A^2$ mating type of this heterothallic, sexually reproducing species was thought to be confined to central Mexico where it was isolated as frequently as the $A^1$ mating type (14).

A retrospective study reported in 1991 indicated that single isolates of the $A^2$ mating type had been recovered in the late 1980s in Pennsylvania and British Columbia (6). These were the first reports of the occurrence of the $A^2$ mating type in natural fungal populations in North America, outside of Mexico. Although the commonly occurring $A^1$ mating type was isolated in these same locations, sexual spores (oospores) were not recovered. In an independent study of mating types in *P. infestans* populations in the United States and Canada, Goodwin *et al.* (15, unpublished results) found that the $A^2$ mating type was rare and not widely distributed; of 178 isolates, only 2 isolates from a single field in British Columbia were found to be $A^2$.

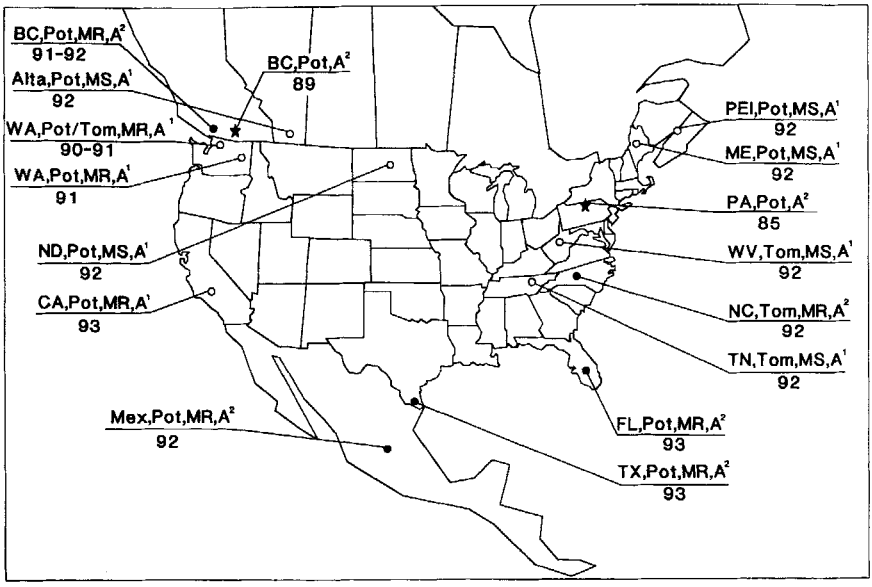


FIG. 1. Collection sites of *Phytophthora infestans* in North America from 1985-1993 (6,8). Location - state or province; Host - potato or tomato; Average metalaxyl response - sensitive (MS) or resistant (MR); Isolation of mating type $A^1$ or $A^2$. • - Sites of $A^2$ isolations ★ - Location of first $A^2$ isolations in the United States and Canada.

Discovery of the rare $A^2$ mating type in North America followed its recovery from other sites outside of Mexico during the mid 1980s. The initial report in 1984 of the existence of $A^2$ isolates in Switzerland (17) prompted extensive sampling in other potato growing regions; results of these studies showed the $A^2$ mating type to be widely distributed in several countries in Europe and the Middle East (5, 16, 17, 21, 27, 30), however, at a low frequency (27). More recently, workers in Japan have reported the sudden appearance of the $A^2$ mating type, as well as a rapid increase in its isolation frequency (22, 23). They also recovered viable oospores from whole plants or plant tissues that had been experimentally inoculated with paired isolates of compatible mating type that were derived from the same growing region, thus demonstrating the population's capacity to become sexual (23).

Despite co-existence of both mating types in central Mexico, field collections of *P. infestans* in northern Mexico from 1950 to 1970 had yielded only the $A^1$ mating type (24). More recent analysis of genetic structure in populations of *P. infestans* from northern Mexico has revealed the existence of both mating types in this area (15). In light of the fairly recent spread of the $A^2$ mating type to northern Mexico, further spread to the United States seemed probable.

Collaborative surveys of various sites of commercial potato production, as well as home garden tomatoes, in the United States, Canada, and Mexico

TABLE 1.—*Origin and collection date of* Phytophthora infestans *isolates evaluated in this study.*

| Location | Collection Date | Host | No. of isolates | Reported severity[a] | Collector |
|---|---|---|---|---|---|
| *United States* | | | | | |
| Florida | 2/93 | Potato | 19 | light-severe | P. Weingartner |
| California | 6/93 | Potato | 35 | light-spotty | R. Hoopes |
| Maine | 7/92 | Potato | 5 | light | R. Goth |
| North Carolina | 7/92 | Tomato | 2 | moderate | P. Shoemaker |
| North Dakota | 8/92 | Potato | 21 | moderate-severe | N. Gudmestad, G. Secor |
| Tennessee | 8/92 | Tomato | 3 | moderate | N. Lapp |
| Texas | 3/93 | Potato | 68 | moderate | J. Wallace |
| Washington | 8/91 | Potato | 28 | moderate | D. Inglis, G. Pelter, O. Riberio |
| West Virginia | 8/92 | Tomato | 3 | moderate | R. Young |
| *Canada* | | | | | |
| Alberta | 8/92-3/93 | Potato | 43 | high-severe | R. Howard |
| British Columbia | 9/91 | Potato | 30 | moderate-high | G. McCollum, D. Ormrod |
| Prince Edward Island | 8/92 | Potato | 45 | moderate-severe | B. Platt, M. Clark, R. Reddin |
| *Mexico* | | | | | |
| Chapingo | 8/92 | Potato/wild *Solanum* spp. | 48 | severe | K. Deahl, M. Cadena |
| Toluca | 8/92 | Potato/wild *Solanum* spp. | 63 | severe | K. Deahl, A. Rivera-Pená |

[a]Collectors' estimate of late blight severity on host plants from which the sample was taken (light-severe).

were undertaken to determine the incidence of metalaxyl resistance in field populations of *P. infestans*. We have attempted also to determine the frequency of the $A^2$ mating type in these populations, and to detect any correlations among metalaxyl response and mating type reactions.

## Materials and Methods

*Quarantine Precautions*—Isolates from Mexico were imported into the United States under strict quarantine guidelines from the U.S. Department of Agriculture (USDA). All *in vitro* studies were conducted in a laminar-flow, biosafety cabinet (Class II, Type A) in an approved quarantine laboratory. All materials were autoclaved at the end of each experiment. *In vivo* inoculations using sterile plant tissues were performed only with isolates from the U.S.

*Isolation From Naturally-Infected Material*—Isolates were obtained from commercial potato crops, potato storages, home gardens, and wild *Solanum* spp. (Table 1). Many of those obtained from commercial potatoes were collected from plants that had received several applications of metalaxyl, although precise records of fungicide treatment were incomplete. From each sample of late blight, one (or more) isolates of *P. infestans* was established in pure culture, each taken from a single lesion on infected potato or tomato foliage or stems, potato tubers, or tomato fruits. Infected tissues were surface-sterilized in 0.5% NaOCl for three minutes, blotted on a sterile paper towel, rinsed in sterile deionized water, and plated on rye agar medium (see below). The isolation plates were incubated at 15-16 C in darkness for 48-72 hours. Spores and mycelia from the infected tissue pieces were examined with the aid of a dissecting microscope, then transferred to fresh rye medium amended with antibiotics [100 ppm each of penicillin G, pimaricin, and polymyxin (25)] and incubated at 18-21 C in darkness. Agar plugs (4 mm diam) containing each fungal isolate were transferred to unamended rye grain agar to initiate pure cultures.

*Culture Maintenance*—*P. infestans* isolates were maintained at 18-21 C in darkness on rye grain agar medium prepared by steaming rye seed (100 g/L deionized water) for 25-30 min, with 1.5% agar added to the resulting broth filtrate. Mycelial mass transfers to fresh media were made at 30-day intervals with plugs cut from the outer zones of active hyphal growth. Some isolates were also maintained on rye agar medium containing 0.05% glucose, because added glucose improved growth.

*Determination of Mating Reaction*—The mating (or compatibility) type of each isolate was determined by centrally inoculating plates of rye grain agar with a mycelial plug (5 x 5 mm) from the isolate of unknown mating type; mycelial plugs from known $A^1$ and $A^2$ isolates were each transferred to opposite sides (at a distance of 2 cm) of the unknown strain. Test plates were incubated at 18-21 C for 10-15 days in darkness and examined microscopically for the presence of gametangia (oogonia with amphigynous antheridia containing mature oospores) at the interface of the advancing colonies. When gametangia were formed in the $A^2$ pairing, the isolate was rated as an $A^1$; when gametangia were formed in the $A^1$ pairing, the unknown isolate was rated as an $A^2$. To eliminate the possibility that gametangial formation was the result of mating between the known test strains, oospore formation was confirmed by single pairings with known $A^1$ or $A^2$ strains. All isolates were mated at least twice, and tester isolates were mated periodically with each other to verify compatibility type. Some isolates were also evaluated on rye-glucose or rye-lima bean agar, when these media improved growth.

In Vitro *Response to Metalaxyl*—*In vitro* growth response of each isolate to metalaxyl was assessed by comparing radial mycelial growth in metalaxyl-amended rye agar media to growth in metalaxyl-free controls. Metalaxyl 2E (Ridomil®) containing 25.1% active ingredient (a.i.) was added to por-

TABLE 2.—*Metalaxyl response of North American strains of* Phytophthora infestans *isolated during 1991-93.*

| Location | Metalaxyl Responses | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | Sensitive | | Intermediate | | Resistant | | |
| Canada | | | | | | | |
| Alberta | 32 | (84)[a] | 6 | (16) | 0 | (0) | 38 |
| British Columbia | 4 | (13) | 8 | (27) | 18 | (60) | 30 |
| Prince Edward Island | 42 | (93) | 3 | (7) | 0 | (0) | 45 |
| Mexico | | | | | | | |
| Chapingo | 1 | (2) | 11 | (23) | 36 | (75) | 48 |
| Toluca | 16 | (25) | 39 | (62) | 8 | (13) | 63 |
| United States | | | | | | | |
| California | 0 | (0) | 2 | (6) | 32 | (94) | 34 |
| Florida | 7 | (39) | 1 | (6) | 10 | (56) | 18 |
| Maine | 5 | (100) | 0 | (0) | 0 | (0) | 5 |
| North Carolina[b] | 0 | (0) | 0 | (0) | 2 | (100) | 2 |
| North Dakota | 21 | (100) | 0 | (0) | 0 | (0) | 21 |
| Tennessee[b] | 3 | (100) | 0 | (0) | 0 | (0) | 3 |
| Texas | 14 | (21) | 3 | (4) | 50 | (75) | 67 |
| Washington | 0 | (0) | 1 | (4) | 27 | (96) | 28 |
| West Virginia[b] | 3 | (100) | 0 | (0) | 0 | (0) | 3 |
| Totals | 148 | | 74 | | 183 | | 405 |

[a]Percent of total.
[b]Isolates from infected tomatoes.

tions of molten rye seed agar prior to autoclaving, to yield final concentrations of 1.0, 10, and 100 μg/ml. Agar medium was prepared as described above, with addition of 0.05% glucose. For each test, inocula consisted of agar plugs (5 mm diam) cut from the outer zones of active growth from cultures aged 10-20 days. Three plugs were placed equidistant on fresh media with or without metalaxyl amendment, followed by incubation at 18-21 C in darkness. Growth was measured typically after 6-7 days, except for slower growing isolates that were tested once radial growth had reached 25 mm or more, usually with 2-3 days additional incubation. *P. infestans* control strains included with each set of tests were metalaxyl standards 575 (metalaxyl-sensitive, MS) and 1100 (metalaxyl-resistant, MR) [obtained from W. Fry, Cornell University]; plus MEX-178, a resistant Mexican isolate. *In vitro* growth response for each isolate was calculated according to the following equation:

$$\text{Percent growth} = \frac{\text{avg diam (-5 mm) on metalaxyl-containing media}}{\text{avg diam (-5 mm) on metalaxyl-free media}} \text{ (X 100\%)}$$

All *P. infestans* isolates, including controls, were designated as metalaxyl-resistant (MR), -intermediate (MI), or -sensitive (MS) according to Shattock (26), based on percent growth in the presence of 10 μg metalaxyl/ml, relative to the metalaxyl-free control. Isolates classified as MR, MI, or MS exhibited >60%, 10-60%, or <10% growth, respectively.

In Vivo *Mating Reaction*—To determine if isolates from selected *P. infestans* populations had the potential to become sexual, sterile potato plants (cv. Katahdin, R0) were inoculated with isolates of compatible mating types ($A^1$ x $A^2$) that had been isolated from the same potato growing area. Asexual sporangia consisting of 1:1 mixtures of each mating type were inoculated onto detached leaflets placed on rye grain agar, then incubated under low-light conditions at 15 C. Infected tissues were examined for the presence of oospores beginning five days after inoculation.

## Results and Discussion

During 1991 to 1993, a total of 413 *P. infestans* isolates from the United States, Canada, and Mexico were characterized for metalaxyl sensitivity and mating type. The geographic origin, collection date and source, host plant, and reported late blight severity that prevailed in collection sites are indicated in Table 1. During this period, several states or provinces had experienced outbreaks of late blight that, according to cooperators' evaluations, varied in severity from mild damage to severe epidemics in areas where conditions were highly favorable to disease development.

*Metalaxyl Testing*—There was marked variation in metalaxyl response from isolates obtained from different geographic locations (Table 2). From Prince Edward Island, 42/45 isolates were metalaxyl-sensitive (MS), as were all 5 isolates from Maine. Isolates from PEI were obtained from potato plants that had received several applications of metalaxyl, yet only 3/45 isolates yielded low level (*i.e.*, designated as metalaxyl-intermediate, MI) resistance. Thirty-two of 38 isolates from Alberta, Canada were MS, and the rest were MI. Twenty-one of 21 isolates from North Dakota were MS. Isolates from these four sites were collected during Summer 1992, and in the case of Alberta, through Spring of the following year.

Other samples yielded primarily MR, or MR and MI isolates. Among 30 isolates collected in British Columbia in 1991, 18 were MR, and 26 were either MR or MI. Thirty-four of 34 isolates from California were MR or MI; most (32/34) were MR. Among Texas isolates, 53/67 were MI or MR, and 50/67 were MR. Eleven of 18 isolates from Florida were MR or MI, and the majority (10/18) were MR. Isolates from California, Texas, and Florida were collected during Spring and Summer 1993. Smaller samples were obtained in Summer 1992 from additional sites of late blight infection in the eastern United States, including North Carolina, Tennessee, and West Virginia. Unlike the isolates mentioned thus far, which were collected from infected

TABLE 3.—*Mating types of North American strains of* P. infestans *isolated during 1991-93.*

| Location | Mating Type | | | | |
|---|---|---|---|---|---|
| | $A^1$ | (%) | $A^2$ | (%) | Total |
| Canada | | | | | |
| Alberta | 38 | (100)[a] | 0 | (0) | 38 |
| British Columbia | 28 | (93) | 2 | (7) | 30 |
| Prince Edward Island | 45 | (100) | 0 | (0) | 45 |
| Mexico | | | | | |
| Chapingo | 10 | (21) | 38 | (79) | 48 |
| Toluca | 9 | (14) | 54 | (86) | 63 |
| United States | | | | | |
| California | 35 | (100) | 0 | (0) | 35 |
| Florida | 7 | (37) | 12 | (63) | 19 |
| North Carolina[b] | 0 | (0) | 2 | (100) | 2 |
| North Dakota | 21 | (100) | 0 | (0) | 21 |
| Maine | 5 | (100) | 0 | (0) | 5 |
| Tennessee[b] | 3 | (100) | 0 | (0) | 3 |
| Texas | 15 | (22) | 53 | (78) | 68 |
| Washington | 28 | (100) | 0 | (0) | 28 |
| West Virginia[b] | 3 | (100) | 0 | (0) | 3 |
| Totals | 247 | | 161 | | 408 |

[a]Percent of total.
[b]Isolates from infected tomatoes.

potatoes, these were obtained from tomatoes, from either commercial plantings in the case of North Carolina and Tennessee, or from home gardens in West Virginia. Six isolates from Tennessee and West Virginia, three from each state, were MS. Two isolates from North Carolina were MR.

MS, MI, and MR phenotypes were isolated from two sites in central Mexico (Table 2). From Chapingo, 47/48 were MR or MI, and 36/48 were MR. Samples from Toluca yielded a higher proportion of sensitive or intermediate isolates; 16/63 were MS, and 39/63 were MI. The sampling sites at Toluca and Chapingo are 75-100 km apart and are separated by high mountain ranges, thus the isolates collected from these two locations probably did not originate from the same population (15).

*$A^2$ Frequency*—Most Canadian isolates were $A^1$ mating type (Table 3). Of 113 isolates collected from 3 locations in eastern and western Canada, only 2 isolates from British Columbia were $A^2$ mating type. These were collected from diseased potato plants in an area nearby the site where the first $A^2$ mating type was found in Canada in 1989 (6).

In central Mexico, the $A^2$ mating type was isolated 4-5 times more frequently than was the $A^1$ mating type. The frequencies of $A^2$ isolation were 38/48 for Chapingo, 54/63 for Toluca, and 92/111 overall. In a genetic analysis of the *P. infestans* population in central Mexico, Goodwin *et al.* found nearly equivalent numbers of $A^1$ and $A^2$ mating types in central Mexico (15). This ratio verified the findings of Gallegly and Galindo (14), who first discovered two *P. infestans* mating types in the Toluca Valley, and who had postulated the existence of nearly equivalent numbers for a fungal population that was primarily reproducing by sexual means. More testing must be done to determine whether our findings of higher frequencies of $A^2$ mating types in this area indicate that these isolates may be more fit.

The high frequency of MR isolates found in field isolations from Mexico in this investigation suggests also the need for additional sampling and study. Others have found that MR field isolates of *P. infestans* have a higher degree of fitness than MS isolates (19, 20). In these studies of Israeli *P. infestans* isolates, undertaken because late blight outbreaks had become more frequent and severe in a five-year period following initial detection of metalaxyl resistance, a composite fitness index was computed from several fitness components including lesion area, infection frequency, and sporulation capacity. Others have also observed that metalaxyl resistant strains from Mexico (15) and Scotland (18) have an equal or a higher fitness in potato leaves and tubers.

Significant numbers of $A^2$ mating types were isolated as well from several collection sites in the United States. From Florida, 12/19 isolates were $A^2$ mating type, and 53/68 Texas isolates were $A^2$s (Table 3). These isolates were collected in Spring 1993 from infected potato plants in two Texas locations and from several counties in Florida. From these sites, $A^1$ and $A^2$ mating types were sometimes isolated from the same infected field. Two $A^2$ isolates were obtained also from infected tomato plants in North Carolina in Summer 1992.

Other collection sites yielded only $A^1$ mating types. Only $A^1$ isolates were obtained from Washington (28 total) in 1991, and from North Dakota (21), West Virginia (3) and Maine (5) in 1992. Similarly, only $A^1$s were obtained from 35 California isolates in Summer 1993. Overall, 67 of 184 isolates collected in the United States during the period of this study were $A^2$ mating types, and 117 of 184 isolates were $A^1$ mating types.

*$A^2$ Origin*—We reported in 1991 the isolation of the $A^2$ mating type from a collection site in Pennsylvania, which was the first published report of the $A^2$ in the United States (6). This single isolate came from infected potatoes in a home garden in that state. Additional $A^2$s have been isolated in subsequent testing in the New York area (Fry, personal communication). As indicated previously, other workers have reported during the past decade the isolation of the $A^2$ outside of Mexico, the site of origin of the *P. infestans*: potato pathosystem; furthermore, Shattock *et al.* (27) have postu-

TABLE 4.—*Metalaxyl response and mating type of* Phytophthora infestans *isolates recovered from late blight-infected* Solanum *spp. in central Mexico.*

| Solanum *species* | Number of isolates | | | | |
|---|---|---|---|---|---|
| | Metalaxyl Response[a] | | | Mating Type | |
| | S | I | R | $A^1$ | $A^2$ |
| *S. verrucosum* | 0 | 4 | 4 | 0 | 8 |
| *S. stoloniferum* | 0 | 0 | 8 | 0 | 8 |
| *S. demissum* | 0 | 8 | 6 | 7 | 7 |
| *S. iopetalum* | 0 | 0 | 3 | 0 | 3 |
| *S. edinense* | 0 | 0 | 4 | 4 | 0 |

[a] Classified as Resistant (R), Intermediate (I), or Sensitive (S), based on percent growth on media containing 10 μg metalaxyl/ml versus metalaxyl-free control. R = >60% growth, I = 10-60% growth, and S = <10% growth.

lated that the $A^2$ may have existed elsewhere at low, undetectable levels. Since only limited sampling of the mating type frequencies in fungal populations in the United States has been done during this period, it is not clear whether the $A^2$ has also been present, prior to the initial report of its recovery.

Long-lived, resistant oospores resulting from sexual mating between compatible mating types have been identified in plant tissues in Mexico, where the two sexual types co-exist (13, 14). Thus, co-isolation of the two mating types in natural fungal populations signifies that sexual mating may occur, and therefore, development of new, more virulent fungal races is possible. Our findings indicate that $A^2$ mating types exist in high frequencies in certain areas of the United States. Multiple sampling from specific sites, along with virulence testing, has not been done, but would help to determine if genetic changes, with development of enhanced virulence profiles, are occurring in these areas.

*Comparison of Mating Type and Metalaxyl Response*—Fungal isolates from late blight samples from central Mexico included isolates from natural infections in wild *Solanum* species growing nearby in commercial potato research plots. Metalaxyl responses and mating reactions for 37 isolates obtained from 5 wild potato species in Toluca and Chapingo are shown in Table 4. All 37 isolates were MR or MI; the majority (25/37) were MR. Since these host plants were not sprayed directly with the fungicide, resistant isolates may have originated nearby in fungicide-treated plantings. Nineteen of 19 isolates that were obtained from *S. iopetalum*, *S. verrocosum*, and *S. stoloniferum* were $A^2$ mating types; no $A^1$s were recovered from these plants. All four isolates from *S. edinense* were $A^1$ mating type. There was an equal ratio of $A^1$ and $A^2$ mating types from *S. demissum*. Overall, 26/37 isolates from wild *Solanum* species were $A^2$ mating type.

TABLE 5.—*Metalaxyl response and mating type among* Phytophthora infestans *isolates from populations from selected locations in North America.*

| Location | Mating Type[a] | Growth as Percent of Control at Three Metalaxyl Concentrations (μg/ml) 100 Avg | 100 Range[b] | 10 Avg | 10 Range | 1 Avg | 1 Range | Metalaxyl Response[c] |
|---|---|---|---|---|---|---|---|---|
| Chapingo | 1 | 72 | 51-88 | 82 | 66-97 | 97 | 81-108 | R |
| | 2 | 47 | 2-106 | 68 | 4-112 | 88 | 14-117 | S/I/R |
| Toluca | 1 | 7 | 0-17 | 28 | 0-47 | 68 | 31-94 | S/I |
| | 2 | 18 | 0-75 | 36 | 0-85 | 64 | 0-135 | S/I/R |
| Texas | 1 | 4 | 0-31 | 3 | 0-25 | 6 | 0-28 | S/I |
| | 2 | 68 | 16-103 | 92 | 44-113 | 99 | 74-140 | I/R |
| Florida | 1 | 0 | 0-0 | 0 | 0-0 | 0 | 0-0 | S |
| | 2 | 60 | 0-104 | 79 | 0-116 | 92 | 0-115 | I/R |
| California | 1 | 95 | 66-246 | 109 | 34-231 | 90 | 26-159 | I/R |
| | 2 | — | — | — | — | — | — | — |
| Prince Edward Island | 1 | 3 | 0-20 | 3 | 0-22 | 5 | 0-27 | S/I |
| | 2 | — | — | — | — | — | — | — |
| Alberta | 1 | 3 | 0-13 | 3 | 0-18 | 5 | 0-23 | S/I |
| | 2 | — | — | — | — | — | — | — |

[a]Sexual mating type - $A^1$ or $A^2$.

[b]Percent growth relative to unamended control, expressed as average value and range of values.

[c]Classified as Resistant (R), Intermediate (I), or Sensitive (S), based on percent growth on media containing 10 μg metalaxyl/ml versus metalaxyl-free control. R = >60% growth, I = 10-60% growth, and S = <10% growth.

Direct comparisons of mating reactions and metalaxyl response patterns among isolates from fungal populations in the United States, Mexico, and Canada are shown in Table 6. Among Mexican isolates from the two collection sites (including isolates from wild potato species), 40/63 from Toluca and 37/48 from Chapingo were both $A^2$ mating type and either MI or MR. Isolates from Chapingo, of both mating types, were more resistant than those from the Toluca Valley; 26/48 of the total sample from Chapingo were MR, compared to 8/63 from the latter site. Chapingo $A^1$ isolates were more resistant, on average, than $A^2$s from this site. Isolates from the Toluca site contained a comparatively higher proportion of sensitive isolates among the $A^2$ population, 14/63 versus 1/48 from Chapingo (Table 6).

TABLE 6.—*Distribution summary of metalaxyl response and mating type among* P. infestans *populations from selected locations.*

| Location | Mating Type | Metalaxyl Response S | Metalaxyl Response I/R[a] |
|---|---|---|---|
| Chapingo | 1 (10)[b] | 0 | 0/10 |
| | 2 (38) | 1 | 11/26 |
| Toluca | 1 (9) | 2 | 7/0 |
| | 2 (54) | 14 | 32/8 |
| Texas | 1 (15) | 14 | 1/0 |
| | 2 (52) | 0 | 2/50 |
| Florida | 1 (7) | 7 | 0/0 |
| | 2 (11) | 0 | 1/10 |
| California | 1 (34) | 0 | 2/32 |
| | 2 (0) | 0 | 0/0 |
| Prince Edward Island | 1 (45) | 42 | 3/0 |
| | 2 (0) | 0 | 0/0 |
| Alberta | 1 (38) | 32 | 6/0 |
| | 2 (0) | 0 | 0/0 |

[a]Number of intermediate isolates/number of resistant isolates.
[b]Total number of each mating type, $A^1$ or $A^2$.

Texas and Florida each yielded a similar correlation between metalaxyl response and mating type, with high numbers of isolates that were MR or MI, and $A^2$ mating type (Table 6). Compared to Mexican samples, the Texas and Florida samples yielded higher frequencies of isolates that were both $A^1$ mating type and MS, 14/67 among Texas isolates and 7/18 among Florida isolates. Among the Mexican isolates, 2/63 from Toluca and 0/48 from Chapingo were $A^1$/MS. Overall, 99-100% of isolates from Florida and Texas were either $A^1$ and MS, or $A^2$ and MI/MR.

A very different pattern was found among California isolates; 34/34 were $A^1$ mating type and 34/34 isolates were MI or MR (Table 6). Among California isolates, the average growth response at 100 µg metalaxyl/ml, relative to unamended control media, was 95% (Table 5). Similarly, Canadian isolates from Alberta and PEI yielded only the $A^1$ mating type. The majority, however, were MS; 32/38 and 42/45 isolates from these sites, respectively, were both $A^1$ mating type and MS (Table 6).

Mycelial growth *in vitro* among California isolates in particular appeared to be stimulated by increasing levels of metalaxyl (Table 5). Average growth responses among 34 resistant isolates were 95%, 109%, and 90% in the presence of 100, 10, and 1.0 µg metalaxyl/ml, respectively. At 100 µg/ml, growth responses among individual isolates ranged to 246% of the growth in unamended control media, and for 12/35 isolates, percent growth exceeded 100%. The reasons for this response are not known, although others have found that metalaxyl may stimulate growth of *Phytophthora in vitro* (2, 11). In one study of *P. parasitica*, increased linear growth in metalaxyl-amended media was not associated with increased biomass in liquid culture, and the single isolate tested was more sensitive to the fungicide in liquid than on solid media (11), indicating that growth media characteristics may influence fungicide resistance measurements.

*Correlation with* In Vivo *Resistance*—We previously reported high level *in vitro* resistance among a collection of 73 isolates from northwestern Washington (7,8), and also discussed some of the limitations of *in vitro* resistance testing (8). In that study, growth response patterns in artificial media were compared with metalaxyl bioassays in excised plant tissues. For most of the 50 isolates tested in both systems (68%), there was good correlation, in terms of overall metalaxyl sensitivity or resistance, between the two test systems. There were, however, significant quantitative differences in the resistance values obtained for a number of isolates. Likewise, other investigators have addressed the validity of metalaxyl response evaluation by testing in artificial media or in plant tissues (1, 3, 26, 27, 28, 29, 32). Most of these studies have shown good correlation between the two test forms, particularly when large sample numbers have been evaluated (26, 27, 28, 32).

In particular, the significance of MI isolates and their role under natural disease conditions remains unclear. Unlike others (26, 27), we failed to detect a clear distinction between MR and MI isolates when multiple assay systems were used (8), although we have found in limited testing that MI isolates possess moderate to high level resistance when tested *in vivo*. In our previous study, 14/73 isolates collected in Washington in 1991 were classed as MI from *in vitro* evaluation. All ten isolates from this group that were also tested on potato leaf disks or tuber disks were resistant to 1-10 µg metalaxyl/ml. Furthermore, 13 MI isolates from the current study, most of them from Maine or Wisconsin, were tested independently by Tom Young [Ciba, Vero Beach, FL]. He found that all were metalaxyl-resistant when tested *in vivo* on excised potato leaves (personal communication).

*Oospore Formation* In Vivo—Finally, we determined whether oospore formation could occur *in vivo* among selected *P. infestans* isolates from Florida and Texas since these particular populations contained both mating types, and in some collection sites, both mating types were isolated from a single infected field. When six each of $A^1$ and $A^2$ mating types were com-

bined in 1:1 ratios of asexual sporangia, oospore initials were formed on the surfaces of sterile potato leaves in five days. Mature oospores were detected within leaf tissues in 10-12 days. Use of sterile plant tissues yielded more consistent results than did attempts to use non-sterile stem and leaf tissues, which, as others have found (6, 12), rapidly deteriorated from secondary bacterial infections. Oospores that were produced in matings between isolates from these two sites appeared to be morphologically identical to those produced *in vitro*. Although it is unclear whether these sexual spores are capable of normal germination and development into viable progeny, occurrence of compatible mating types within these populations suggests that sexual reproduction in natural populations in the field is possible, and also suggests the potential for new genetic segregants with increased metalaxyl resistance.

**Literature Cited**

1. Bruck, R.I., W.E. Fry and A.E. Apple. 1980. Effect of metalaxyl, an acylalanine fungicide, on developmental stages of *Phytophthora infestans*. Phytopathology 70(7):597-601.
2. Bruin, G.C.A. and L.V. Edginton. 1981. Adaptive resistance in *Peronosporales* to metalaxyl. Can J Plant Pathol 3:201-206.
3. Coffey, M.D. and L.W. Young. 1984. Responses to metalaxyl of sensitive and resistant isolates of *Phytophthora infestans*. Phytopathology 74:615-620.
4. Davidse, L.C., D.L. Danial and C.J. Van Western. 1983. Resistance to metalaxyl in *Phytophthora infestans* in the Netherlands. Neth J Plant Pathol 89:1-20.
5. Davidse, L.C., D. Looijen, L.J. Turkensteen and D. Van Der Wal. 1981. Occurrence of metalaxyl-resistant strains of *Phytophthora infestans* in Dutch potato fields. Neth J Plant Pathol 87:65-68.
6. Deahl, K.L., R.W. Goth, R. Young, S.L. Sinden and M.E. Gallegly. 1991. Occurrence of the $A^2$ mating type of *Phytophthora infestans* in the United States and Canada. Am Potato J 68:717-725.
7. Deahl, K.L., D.A. Inglas and S.P. DeMuth. 1991. Testing for resistance to metalaxyl in *Phytophthora infestans* isolates from northwestern Washington. Am Potato J 68:605 (abstract).
8. Deahl, K.L., D.A. Inglas and S.P. DeMuth. 1993. Testing for resistance to metalaxyl in *Phytophthora infestans* isolates from northwestern Washington. Am Potato J 70:779-795.
9. Dowley, L.J. and E. O'Sullivan. 1981. Metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. Potato Res 24:417-421.
10. Dowley, L.J. and E. O'Sullivan. 1985. Monitoring metalaxyl resistance in populations of *Phytophthora infestans*. Potato Res 28:531-534.
11. Ferrin, D.M. and M.L. Wadsworth. 1992. Effect of metalaxyl on sporulation and growth of metalaxyl-resistant and metalaxy-sensitive isolates of *Phytophthora parasitica in vitro*. Plant Dis 76(5):492-495.
12. Frinking, H.D., L.C. Davidse and H. Limburg. 1987. Oospore formation by *Phytophthora infestans* in host tissue after inoculation with isolates of opposite mating type found in the Netherlands. Neth J Plant Pathol. 93:147-149.
13. Galindo, A.J. and M.E. Gallegly. 1960. The nature of sexuality in *Phytophthora infestans*. Phytopathology 50:123-128.
14. Gallegly, M.E. and A.J. Galindo. 1958. Mating types and oospores of *Phytophthora infestans* in nature in Mexico. Phytopathology 48:274-277.

15. Goodwin, S.B., L.J. Spielman, J.M. Matuszak, S.N Bergeron and W.E. Fry. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82:955-961.

16. Grinberger, M., D. Kadish and Y. Cohen. 1989. Occurrence of the $A^2$ mating type and oospores of *Phytophthora infestans* in potato crops in Israel. Phytoparasitica 17:197-204.

17. Hohl, H.R. and K. Iselin. 1984. Strains of *Phytophthora infestans* from Switzerland with $A^2$ mating type behaviour. Trans Br Mycol Soc 83: 529-530.

18. Holmes, S.J.I. and A.G. Channon. 1984. Studies on metalaxyl-resistant *Phytophthora infestans* in potato crops in south-west Scotland. Plant Pathol 33:347-354.

19. Kadish, D. and Y. Cohen. 1988. Fitness of *Phytophthora infestans* isolates from metalaxyl-sensitive and metalaxyl-resistant populations. Phytopathology 78:912-915.

20. Kadish, D., M. Grinberger and Y. Cohen. 1990. Fitness of metalaxyl-sensitive and metalaxyl-resistant isolates of *Phytophthora infestans* on susceptible and resistant potato cultivars. Phytopathology 80(2):200-205.

21. Malcolmson, J.F. 1985. *Phytophthora infestans*: $A^2$ compatibility type recorded in Great Britain. Trans Br Mycol Soc 85:531.

22. Mosa, A.A., M. Kato, N. Sato, D. Kobayashi and A. Ogoshi. 1989. Occurrence of the $A^2$ mating type of *Phytophthora infestans* on potato in Japan. Ann Phytopath Soc Japan 55:615-620.

23. Mosa, A.A., M. Kato, N. Sato, D. Kobayashi and A. Ogoshi. 1990. Oospore formation by *Phytophthora infestans* in potato leaves after inoculation with isolates of opposite mating type. Ann Phytopath Soc Japan 56:144 (abstract).

24. Niederhauser, J.S. 1991. *Phytophthora infestans*: The Mexican connection. *In: Phytophthora*, J.A. Lucas, R.C. Shattock, D.S. Shaw and L.R. Cooke, eds., Cambridge University Press, Cambridge. pp. 25-45.

25. Ribeiro, O.K. 1978. *A Source Book of the Genus Phytophthora*. J. Cramer, Germany. p. 279.

26. Shattock, R.C. 1988. Studies on the inheritance of resistance to metalaxyl in *Phytophthora infestans*. Plant Pathol 37:4-11.

27. Shattock, R.C., D.S. Shaw, A.M. Fyfe, J.R. Dunn, K.H. Loney and J.A. Shattock. 1990. Phenotypes of *Phytophthora infestans* collected in England and Wales from 1985 to 1988: mating type, response to metalaxyl, and isoenzyme analysis. Plant Pathol 39:242-248.

28. Sozzi, D. and T. Staub. 1987. Accuracy of methods to monitor sensitivity of *Phytophthora infestans* to phenylamide fungicides. Plant Dis 71(5):422-425.

29. Staub, T., H. Dahmen, P. Urech and F. Schwinn. 1979. Failure to select for *in vivo* resistance in *Phytophthora infestans* to acylalanine fungicides. Plant Dis Rep 63(5):385-389.

30. Tantius, P.H., A.M. Fyfe, D.S. Shaw and R.C. Shattock. 1986. Occurrence of the $A^2$ mating type and self-fertile isolates of *Phytophthora infestans* in England and Wales. Plant Pathol 35:578-581.

31. Therrien, C.D., P.W. Tooley, L.J. Spielman, D.I. Ritch and S.E. Shelley. 1993. Nuclear DNA content, allozyme phenotypes and metalaxyl sensitivity of *Phytophthora infestans* from Japan. Mycol Research 97(8):945-950.

32. Walker, A.S.L. and L.R. Cooke. 1990. The survival of *Phytophthora infestans* in potato tubers - the influence of phenylamide resistance. Brighton Crop Protection Conference 1990—Pests and Diseases:353-359.