# Fitness of *Phytophthora infestans* Isolates from Metalaxyl-Sensitive and -Resistant Populations

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## ABSTRACT

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Twenty field isolates of *Phytophthora infestans* from different locations in Israel were compared on potato plants in growth chambers, and six isolates were compared on plants grown in walk-in plastic tunnels. Although lesion areas induced in potato plants (cv. Alpha) by metalaxyl-resistant (MR) isolates were significantly ($P < 0.0001$) larger than those induced by metalaxyl-sensitive (MS) isolates, no significant differences were recorded among isolates in sporulation capacity or infection efficiency. Composite fitness indices in growth chambers and epidemic development in plastic tunnels were significantly higher for MR than for MS isolates, thus providing a possible explanation for the severe MR-induced late blight epidemic outbreaks in commercial potato fields in Israel.

*Additional keywords*: epidemiology, fungicide resistance, oomycetes.

Late blight incited by *Phytophthora infestans* (Mont.) de Bary is the most destructive disease of potato in Israel. Disease outbreaks became more frequent and more severe in the five years since metalaxyl-resistant isolates of the fungus were detected in 1982 (4). In 1986, 38 out of 45 isolates collected and, in 1987, 20 out of 24 isolates collected were resistant to phenylamide fungicides (metalaxyl, oxadixyl, and benalaxyl) in spite of the fact that Ridomil-MZ was rarely used. Occurrence of metalaxyl-resistant isolates was frequent also in fields not treated with phenylamide-containing fungicides (2). Although fungicide-resistant fungal mutants may exhibit lower fitness to their respective hosts (9), no such reduced fitness was recorded in phenylamide-resistant oomycete fungal agents (3,6,7).

Comparative examination of some fitness components was conducted in clones and populations of *Plasmopara viticola* (12) and *Bremia lactucae* (7) resistant and sensitive to phenylamide, but detailed studies in other oomycetes are lacking. The objective of this study was to compare fitness components in metalaxyl-sensitive (MS) and metalaxyl-resistant (MR) isolates of *P. infestans*. Ten MS and 10 MR field isolates of the fungus were compared on leaves of potato plants in a constant environment in growth chambers in essentially the same manner described by Tooley et al (14), and three MS and three MR isolates were compared in plastic tunnels in the field. Some of the results reported were published earlier (5).

## MATERIALS AND METHODS

**Plant material.** All experiments were conducted with *Solanum tuberosum* L. 'Alpha.' Plants were grown in the greenhouse (19–28 C) from certified potato tubers in 1.5-L pots containing sandy loam (about 100 g), one tuber per pot. Plants were fertilized twice a week with 1% N:P:K (20:20:20) solution. Plants were used for inoculation tests at 6–7 wk after sowing when they had 3–4 shoots per pot with 9–10 compound leaves per shoot.

**Fungal isolates.** Ten MS and 10 MR isolates of *Phytophthora infestans* were collected from blighted potato fields. Details on the location, cultivar, and date of collection are given in Table 1 and Figure 1.

Isolates were maintained on detached potato leaflets or tuber slices placed on water-saturated filter paper in petri dishes or plastic boxes (20×10×5 cm) at 12 C. The isolates were periodically tested for sensitivity to metalaxyl by inoculating potato tuber disks lying on a filter paper saturated with a solution of metalaxyl (25 WP, 0.01–1,000 mg active ingredient per liter). Details on the isolates' sensitivity to metalaxyl are given in the Results section (Table 2).

**Fitness components.** The following fitness components (14) were comparatively measured in MS and MR isolates: 1) infection frequency = proportion of leaflets infected out of those inoculated; 2) lesion area = areas of lesion produced at 6 days postinoculation in intact leaflets; 3) sporulation capacity = number of sporangia produced per square centimeter of lesion in intact leaflets at 7 days postinoculation.

**Assessment of fitness components.** Three experiments were performed. The same 10 MS and 10 MR isolates were used in all

TABLE 1. Details on isolates of *Phytophthora infestans* used in this study

| Isolate[a] | Location | Collection date | Cultivar |
|---|---|---|---|
| MS1 | Bet-Kama | March 1983 | Unknown |
| MS2 | Nir-Eliyahu | February 1984 | Alpha |
| MS3 | Sufa | March 1986 | Alpha |
| MS4 | Shfayim | December 1986 | Alpha |
| MS5 | Petah-Tikva | February 1987 | Alpha |
| MS6 | Nahal-Oz | March 1987 | Dessirre |
| MS7 | Erez | March 1987 | Dessirre |
| MS8 | Nirim | March 1987 | Nikola |
| MS9 | Kisufim | April 1987 | Spunta |
| MS10 | Ein-Hashlosha | April 1987 | Spunta |
| MR1 | Guvloth | November 1984 | Mirka |
| MR2 | Bror-Hayil | March 1985 | Alpha |
| MR3 | Mishmereth | March 1986 | Unknown |
| MR4 | Zeelim | December 1986 | Unknown |
| MR5 | Magen | January 1987 | Ethica, Mirka |
| MR6 | Ramat-Hasharon | February 1987 | tomato |
| MR7 | Saad | March 1987 | Spunta, Dessirre |
| MR8 | Dorot | March 1987 | Dessirre |
| MR9 | Nir-Yizshak | March 1987 | Alpha |
| MR10 | Beeri | April 1987 | Alpha |

[a] MS = Metalaxyl sensitive; MR = metalaxyl resistant.

three experiments. In each experiment, 60 plants were used. Three plants were inoculated with each isolate. Thirty leaflets (mostly subterminal) were inoculated in each plant totaling 90 leaflets per isolate per experiment. A 10-$\mu$l droplet of sporangial suspension (20,000 sporangia/ml) of a given isolate was placed on the upper surface of the potato leaflet. Inoculated plants were maintained at 100% RH in darkness for 20 hr to allow infection by the pathogen. Plants were then transferred to a growth chamber maintained at 20 C (60–70% RH) with a 12-hr photoperiod (120 $\mu$Einsteins per square meter per second). At 6 days after inoculation, the three fitness components were measured. First, the proportion of inoculated leaflets on which lesions developed was recorded (infection frequency). Second, lesion area was measured in intact infected leaflets (usually about 20–25 per plant). Lesion area was recorded by tracing the lesion perimeter on a transparent millimetric paper and counting the number of square millimeters enclosed within the margins recorded. The third fitness component, sporulation capacity, was measured as follows. After lesions were allowed to grow 6 days, they were again maintained at 100% RH at 20 C for 20 hr to induce fungal sporulation. Individual infected leaflets were then detached, placed in a beaker with 50 ml of Formalin, acetic acid, ethanol (FAA) (5:5:90 by volume), and shaken 5 min to dislodge sporangia. Leaflets were then blotted dry between paper towels, and lesion area was measured again. Sporangial counts in the FAA were taken with the aid of a hemacytometer (5 counts/leaflet). Sporulation capacity per square centimeter of lesion area was calculated by dividing the number of sporangia produced on a leaflet by the area (7th day) of the lesion on this leaflet.

A composite fitness index (CFI) was calculated for each isolate in each of the three experiments as the product of infection frequency, lesion area, and sporulation capacity. CFI gives an indication of the number of progeny lesions produced from a single parent lesion (15).

**Epidemics in plastic tunnels.** Potato crops (cv. Alpha) were grown in 6 plastic tunnels (10 × 6 × 3 m) in autumn 1986. Three hundred plants in 5 rows (60 plants/row) were grown in each tunnel. Plants were sprinkle irrigated daily with about 5 mm of "rain." Six weeks after planting, the plants in each tunnel were spray inoculated (300 sporangia/ml, 500 ml/tunnel) with one of the six isolates: MS1, MS2, MS3, MR1, MR2, or MR3. Disease development was recorded in each tunnel every 2 days for a period of 26 days using a 0–5 visual scale (0 = no disease and 5 = plants completely blighted). Area under disease progress curve (AUDPC) was computed by numeric integration for each isolate for comparison purposes.

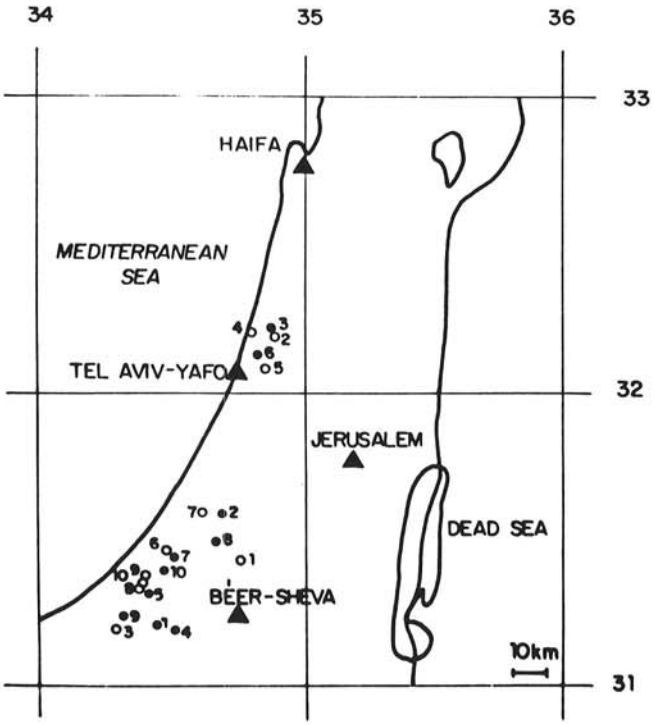


**Fig. 1.** Locations of the farms from which the isolates of *Phytophthora infestans* used in this study were collected. o = metalaxyl-sensitive isolates; ● = metalaxyl-resistant isolates. For details about the isolates, see Table 1.

## RESULTS

The two populations exhibited a large variation for fitness components (Tables 3 and 4). Isolates within each population

TABLE 2. Dosage-response data for the pathogenicity of 20 field isolates of *Phytophthora infestans* to potato tuber disks (cv. Alpha) in the presence of metalaxyl[a]

| Isolate[b] | Tuber disks infected (% ± S.D.) at metalaxyl concentration of:[c] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 0.01 | 0.1 | 1 | 10 | 100 | 1,000 |
| MS1 | 100 | 68 ± 8 | 5 ± 0 | 0 | 0 | 0 | 0 |
| MS2 | 100 | 70 ± 5 | 3 ± 3 | 0 | 0 | 0 | 0 |
| MS3 | 100 | 58 ± 8 | 13 ± 3 | 0 | 0 | 0 | 0 |
| MS4 | 100 | 82 ± 19 | 12 ± 3 | 0 | 0 | 0 | 0 |
| MS5 | 100 | 55 ± 5 | 18 ± 8 | 0 | 0 | 0 | 0 |
| MS6 | 100 | 82 ± 19 | 8 ± 3 | 0 | 0 | 0 | 0 |
| MS7 | 100 | 78 ± 6 | 5 ± 10 | 0 | 0 | 0 | 0 |
| MS8 | 100 | 88 ± 13 | 3 ± 3 | 0 | 0 | 0 | 0 |
| MS9 | 100 | 90 ± 5 | 2 ± 3 | 0 | 0 | 0 | 0 |
| MS10 | 100 | 83 ± 8 | 18 ± 6 | 0 | 0 | 0 | 0 |
| MR1 | 100 | 100 | 100 | 100 | 100 | 98 ± 1 | 10 ± 0 |
| MR2 | 100 | 100 | 100 | 100 | 100 | 100 | 15 ± 0 |
| MR3 | 100 | 100 | 100 | 100 | 100 | 100 | 15 ± 0 |
| MR4 | 100 | 100 | 100 | 100 | 100 | 100 | 13 ± 3 |
| MR5 | 100 | 100 | 100 | 100 | 100 | 100 | 13 ± 3 |
| MR6 | 100 | 100 | 100 | 100 | 100 | 98 ± 3 | 17 ± 3 |
| MR7 | 100 | 100 | 100 | 100 | 73 ± 10 | 27 ± 8 | 0 |
| MR8 | 100 | 100 | 100 | 100 | 100 | 100 | 17 ± 6 |
| MR9 | 100 | 100 | 100 | 100 | 100 | 97 ± 6 | 3 ± 3 |
| MR10 | 100 | 100 | 100 | 100 | 97 ± 3 | 73 ± 8 | 2 ± 3 |

[a] Numbers are averages (± standard deviation) from three experiments with 20 tuber disks/isolate/metalaxyl concentration. Each disk was inoculated with 25 sporangia. Infection was assessed according to the fungal sporulation at 7 days after inoculation.
[b] MS = Metalaxyl sensitive, MR = metalaxyl resistant.
[c] Milligram active ingredient per l.

TABLE 3. Fitness components and composite fitness index for *Phytophthora infestans* field isolates sensitive and resistant to metalaxyl

| Metalaxyl-sensitive (MS) isolates | | | | | Metalaxyl-resistant (MR) isolates | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Isolate | IF[a] | LA[b] | SC[c] | CFI[d] | Isolate | IF | LA | SC | CFI |
| MS1 | 0.78 | 1.41 | 11,083 | 12,189 | MR1 | 0.67 | 7.48 | 5,852 | 29,328 |
| MS2 | 0.77 | 1.11 | 14,317 | 12,237 | MR2 | 0.89 | 4.57 | 17,452 | 70,983 |
| MS3 | 0.84 | 0.86 | 17,079 | 12,338 | MR3 | 0.82 | 6.16 | 9,074 | 45,835 |
| MS4 | 0.82 | 1.04 | 17,266 | 14,904 | MR4 | 0.80 | 5.68 | 17,124 | 77,811 |
| MS5 | 0.95 | 1.36 | 18,078 | 23,357 | MR5 | 0.90 | 5.38 | 16,538 | 82,458 |
| MS6 | 0.92 | 1.55 | 9,548 | 13,615 | MR6 | 0.82 | 4.41 | 10,633 | 38,451 |
| MS7 | 0.9 | 0.85 | 12,219 | 9,347 | MR7 | 0.73 | 4.31 | 9,069 | 28,533 |
| MS8 | 0.82 | 0.85 | 13,964 | 9,732 | MR8 | 0.77 | 4.19 | 17,357 | 55,998 |
| MS9 | 0.82 | 0.95 | 16,946 | 14,724 | MR9 | 0.80 | 5.27 | 5,378 | 22,673 |
| MS10 | 0.88 | 0.87 | 14,329 | 10,970 | MR10 | 0.73 | 4.27 | 4,089 | 12,745 |
| LSD[e] | 0.06 | 0.78 | 4,070 | 9,248 | | 0.06 | 0.78 | 4,070 | 9,248 |

[a] IF (infection frequency) = proportion of inoculated leaflets on which lesions developed.
[b] LA (lesion area) = area ($cm^2$) of lesions produced per leaflet.
[c] SC (sporulation capacity) = number of sporangia produced per square centimeter of lesion.
[d] CFI (compound fitness index) = IF × LA × SC.
[e] Least significant difference ($P = 0.05$) for comparing means of isolates both within and between MS and MR populations.

TABLE 4. Means and contrasts between means of metalaxyl-sensitive (MS) and metalaxyl-resistant (MR) field populations of *Phytophthora infestans* for fitness components and composite fitness index

| Fitness measure[a] | Means ± S.D.[a] | | Resistant vs. sensitive population | | |
|---|---|---|---|---|---|
| | MR | MS | Contrast estimate[b] | $t$ | $P > t$ |
| IF[c] | 0.79 ± 0.07 | 0.85 ± 0.06 | −0.06 | 1.88 | 0.0757 |
| LA[d] | 5.17 ± 1.05 | 1.09 ± 0.26 | 4.08 | 11.11 | 0.0001 |
| SC[e] | 11,257 ± 5,406 | 14,482 ± 2,883 | −3,225 | 1.67 | 0.1185 |
| CFI[f] | 46,481 ± 24,338 | 13,341 ± 3,985 | 33,140 | 4.24 | 0.0020 |

[a] Means ± standard deviations from three experiments with 10 isolates per population and 30 leaflets per isolate per experiment.
[b] The contrast estimate represents the difference between the MR and MS populations.
[c] IF (infection frequency) = proportion of inoculated leaflets on which lesions developed.
[d] LA (lesion area) = area ($cm^2$) of lesions produced per leaflet.
[e] SC (sporulation capacity) = number of sporangia produced per square centimeter of lesion.
[f] CFI (compound fitness index) = IF × LA × SC.

differed significantly ($P < 0.05$) in infection frequency, lesion area, and sporulation capacity (Table 3).

Contrast estimates computed for each fitness component (Table 4) revealed that isolates from the MR population had a significantly ($P < 0.0001$) higher lesion area than those from the MS population. In fact, the average lesion area produced by MR isolates was about five times larger than that produced by the MS isolates. The two populations, however, did not differ significantly (at a 5% level) in sporulation capacity or in infection efficiency.

A significant difference ($P < 0.002$) was recorded between the two populations for the compound fitness index (Table 4).

Epidemics conducted in walk-in plastic tunnels revealed some variation among isolates of each population (Fig. 2). However, disease progressed faster in MR-inoculated tunnels than in MS-inoculated tunnels. AUDPC values for MS1, MS2, and MS3 were 42, 26, and 43, respectively, as compared to 70, 75, and 66 for MR1, MR2, and MR3, respectively. A significant difference ($P = 0.05$) was recorded in AUDPC between the two populations.

## DISCUSSION

We found that, in the absence of selection pressure, MR isolates of *P. infestans* have higher compound fitness indices than MS isolates. The MR and MS isolates differed significantly for lesion area but not for sporulation capacity or infection efficiency. Our in vitro studies (Kadish and Cohen, *unpublished*) showed that MS and MR isolates did not significantly differ ($P = 0.05$) in their linear growth or artificial media. Studies made in walk-in plastic tunnels showed that epidemics induced by MR isolates progressed significantly faster as compared to those induced by MS isolates. The presented data collected in growth chambers and the field may explain why outbreaks of late blight due to MR isolates in Israel are frequent and severe in spite of the lack of a positive selection pressure in many fields (2). Although resistance to some fungicides is linked to reduced fitness (9), no such linkage was reported for oomycetes resistant to phenylamide fungicides in general (3) or for *P. infestans* in particular (4,5,8,10).

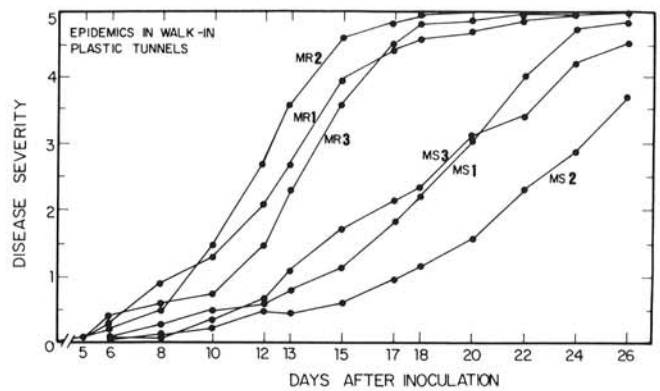


**Fig. 2.** Progress curves of late blight in potatoes (cv. Alpha) induced in six plastic tunnels in autumn 1986 by three metalaxyl-sensitive (MS1, MS2, MS3) and three metalaxyl-resistant (MR1, MR2, MR3) field isolates of *Phytophthora infestans*.

The observation that MR field isolates of *P. infestans* have an equal or a higher fitness in potato leaves and tubers was made by other workers (8,10). However, these studies were done by measuring the proportion of MR sporangia recovered from the population after mixed MR:MS sporangial inoculations, and not by measuring single fitness components. A high degree of competitiveness of MR isolates in the absence of phenylamide fungicides also was observed in *Pseudoperonospora cubensis* in cucumbers and muskmelons (6), *Phytophthora capsici* (1) and *Phytophthora citricola* (11) in peppers and soil, and *Pythium aphanidermatum* in ryegrass (13).

Piganeau and Clerjeau (12) showed that competitiveness of MR isolates of *Plasmopara viticola* on grape leaves is temperature-dependent; although MR strains were more competitive than MS strains in cold conditions (10–22 C), the opposite was true at warm conditions (22–30 C). This difference resulted from a corresponding difference in spore germination and sporulation of the pathogen.

The evidence presented here approximates a higher noncompetitive fitness for MR field isolates of *P. infestans* in

comparison with MS field isolates. Fitness measurements were taken at constant environments in growth chambers or in walk-in plastic tunnels, and they do not include other fitness components, such as oospore production, overwintering, and oversummering, potentially important under field conditions. The high frequency of MR *P. infestans* that we found in tubers sampled in ports before export (Y. Cohen, *unpublished*) calls for studying fitness on tuber tissue as well. Because a high noncompetitive fitness does not necessarily mean a high competitive fitness, we have studied competitive fitness of six isolates in mixed MS:MR inoculations in leaf and tuber tissue at a constant environment in growth chambers and in walk-in plastic tunnels in the field. Those results will be published separately.

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