

Occurrence of Metalaxyl-Resistant Isolates of *Phytophthora infestans* in Potato Fields in Israel

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ABSTRACT

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Isolates of *Phytophthora infestans* resistant to metalaxyl were collected from potato fields in the spring of 1982. An isolate collected 27 April from Nahal-Oz (northwestern Negev) was infective on potato plants drenched with up to 0.5 mg a.i. of metalaxyl per plant, whereas isolates collected from the same location at 2 wk or later were infective on plants drenched with up to 5 mg a.i. of metalaxyl per plant. Disease induced by nine metalaxyl-

sensitive isolates of the pathogen collected during 1977-1981 was controlled in plants drenched with 0.05 mg a.i. of metalaxyl per plant. Growth chamber studies showed that after four infection cycles, conducted on plants treated with increasing dosages of the fungicide, the isolate collected on 27 April was infective on plants drenched with 15 mg a.i. per plant.

The failure of metalaxyl (DL-methyl *N*[2,6-dimethylphenyl]-*N*-methoxyacetyl alanine methyl ester) to control plant diseases incited by fungal pathogens classified among the Peronosporales was repeatedly reported from various parts of the world. Resistance to the fungicide was first reported for *Pseudoperonospora cubensis* in Israel (9) and Greece (8); these were followed by similar reports for *Peronospora hyoscyami* in the U.S. (1), *Phytophthora infestans* in Holland (7) and Northern Ireland (4), and recently for *Plasmopora viticola* in France (5).

One of the measures undertaken by the manufacturer (Ciba-

Geigy Corp.) to reduce the occurrence of metalaxyl-resistant populations was to market the fungicide in a mixture with a protective fungicide (7.5% metalaxyl + 56% mancozeb in Israel and Northern Ireland, 10% metalaxyl + 48% mancozeb in Holland, and 20% metalaxyl + 40% folpet in France). This technique was ineffective and populations resistant to the fungicide mixtures have appeared in Holland (7), Northern Ireland (4), France (5) and now in Israel.

MATERIALS AND METHODS

Potato plant samples infected with *Phytophthora infestans* were collected from experimental fields in Nahal-Oz (northwestern Negev) during the spring of 1982. An area of 34 hectares (ha) was sown on 22 February 1982 with six potato (*Solanum tuberosum* L.)

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TABLE 1. Growth responses^a of isolates of *Phytophthora infestans*, collected on various dates from potato fields, on potato leaflets detached from plants drenched with metalaxyl

Metalaxyl concentration (mg a.i./plant) ^b	1977-1981		27 April 1982		11 May 1982		24 May 1982	
	Lesion diameter (cm)	Sporulation index ^c	Lesion diameter (cm)	Sporulation index	Lesion diameter (cm)	Sporulation index	Lesion diameter (cm)	Sporulation index
0.0	3.7	4	4.2	4	5	4	5	4
0.05	0.8	0	5	4	5	4
0.25	0.6	0	1.6	2	5	4	5	3
0.50	0.4	0	1.2	1.5	5	4	4.7	4
1.25	... ^e	5	4	5	4
2.5 ^d	0.9	0.5	5	4	5	4
5.0 ^d	0.5	0.5	5	3.5	5	3

^a Numbers represent mean values in two different inoculation tests with 100 leaflets per isolate per dose.

^b Potato plants growing in 15-cm-diameter plastic pots (1 kg soil) were drenched with 20 ml metalaxyl suspension 48 hr before leaflets were detached for inoculation.

^c 0 = no visible sporulation; 4 = heavy sporulation.

^d Plants show toxic effects due to fungicide application.

^e Not tested.

TABLE 2. Disease severity index and sporangia production^a of *Phytophthora infestans* isolated 11 May or 24 May from a potato field that had been sprayed with mancozeb and metalaxyl

Metalaxyl concentration (µg a.i./ml)	11 May 1982		24 May 1982	
	Disease index ^b	Sporangia produced per disk (x10 ³) ^c	Disease index	Sporangia produced per disk (x10 ³)
0	4	159 ± 40 A	4	120 ± 32 A
0.25	4	182 ± 40 B	4	129 ± 28 A
0.5 ^d	4	151 ± 36 A	4	112 ± 28 A
2.5	4	93 ± 36 C	4	128 ± 36 A
5.0	4	137 ± 44 A	4	158 ± 35 B
25	4	143 ± 30 A	4	90 ± 20 C
50	4	133 ± 52 A	4	80 ± 18 C
250	4	60 ± 24 D	3.5	54 ± 16 D
500	3.5	24 ± 19 E	3	42 ± 16 D

^a Ten 2-cm-diameter disks per treatment floating on 10 ml of metalaxyl suspension (25 WP) in petri dishes. Isolates were evaluated in separate experiments.

^b Numbers represent necrosis of discs (0-4 visual scale: 0 = no necrosis; 1, 2, and 3 = necrosis occupying about 25, 50, and 75% of the disk area; and 4 = total necrosis).

^c Mean ± standard deviation of the mean. Figures followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

^d Wild-type isolates collected during 1977-1981 were controlled by 0.5-1.0 µg a.i. metalaxyl per milliliter.

cultivars: 5.3 ha with cultivars Croft and Cardinal (extremely susceptible to late blight), 3.7 ha with cultivars Diamant, Alfa, and Spunta (moderately susceptible), and 25 ha with cultivar Desirée (resistant). Seed tubers of Cardinal were imported from Holland and those of Croft from Scotland. Natural infection with late blight was first seen on 30 March. The field was sprayed with mancozeb (2.5 kg of technical product per hectare) on 11 April, and with metalaxyl-mancozeb (3.0 kg technical product per hectare) on 25 April and 9 May. Disease was recorded on, and infected leaf samples were collected from, cultivars Croft and Cardinal on 27 April, 11 and 24 May 1982. Disease was rated by counting numbers of infected leaflets per plant, two plants per site, 20 sites per cultivar, totaling 80 plants at each date.

Five hundred lesioned leaves were sampled from each of the two cultivars at each sampling date. Infected leaves were brought to the laboratory, washed, and placed in moist containers to induce sporulation by *P. infestans*. Sporangia were collected, washed, and suspended in distilled water; their concentration was adjusted to 50,000 sporangia per milliliter and used for inoculation of potato leaves (cultivar Up-to-date). Intact plants (3), detached leaflets or leaf disks (7) were inoculated. Inoculation was done by placing 5-µl

droplets of inoculum suspension of the abaxial surface of the leaf tissue. Experiments were repeated twice or more for each sampling date with either four intact plants or 100 detached leaflets per treatment. The leaf disk test was run once with 10 disks per treatment. Inoculated intact plants were kept in a dew chamber in the dark (15 C) for 20 hr, then transferred to a 15 C cabinet (40-60% RH) illuminated 12 hr/day with cool-white fluorescent lamps (about 80 µE·m⁻²·s⁻¹). Detached leaflets and leaf disks were constantly kept in moist plastic trays or petri dishes under the same conditions. Disease development was recorded at 6-7 days after inoculation by measuring lesion diameter and visually assessing sporulation intensity (0-4 scale: 0 = no sporulation, 4 = abundant sporulation). In some experiments, individual leaflets or leaf disks were transferred (on the seventh day after inoculation) to a known volume of a fixative solution, and sporangia were counted with the aid of a cytometer (four counts per leaflet or leaf disk).

Metalaxyl (a wettable powder containing 25% metalaxyl) application was done as follows: When whole plants were inoculated, the fungicide was applied as a soil drench or foliar spray to runoff; when detached leaflets were inoculated, soil drenches were employed. With either inoculation technique, plants or leaves were inoculated 2 days after a soil drench, and 1 day after a foliar spray. When leaf disks were used, the leaf disks were floated on an aqueous suspension of the fungicide in petri dishes.

RESULTS

The late blight fungus was first observed in the experimental fields in Nahal-Oz on 30 March. Average late blight occurrence on the susceptible potato cultivars, Cardinal and Croft, on 27 April, 11 May, and 24 May was about 0.1, 1.0, and 100 infected leaflets per plant, respectively, for both cultivars. There were 500-600 leaflets per plant.

Isolates of *P. infestans* from leaflets collected on 27 April, 11 May, and 24 May were infective (produced lesions of ≥1 cm with sporulation intensity of ≥1) to leaflets detached from plants that had been drenched with 0.5, 5.0 and 5.0 mg a.i. of metalaxyl per plant, respectively (Table 1). Nine isolates of *P. infestans* collected from potato fields during 1977-1981 and tested at that period were not infective on plants drenched with 0.05 mg a.i. of metalaxyl per plant (Table 1; see also [3]). Table 2 represents the infectivity of the isolates collected on 11 and 24 May to leaf disks floated on metalaxyl. Both isolates produced lesions and sporulated on leaf disks floated on metalaxyl at a concentration 500-1,000 times higher than that required to control disease incited by the wild-type isolates. The 11 May isolate tolerated intermediate concentrations of the fungicide more than the 24 May isolate, whereas the latter isolate was more tolerant of the highest concentrations used than was the former isolate. Sporulation was significantly ($P < 0.05$) stimulated by metalaxyl of 0.25 and 5.0 µg/ml in the 11 May and 24

May isolates, respectively.

The isolate collected 24 May was propagated for four generations on metalaxyl-free plants, and thereafter used for inoculation of leaflets detached from plants drenched with increasing dosages of the fungicide. At 6 days after inoculation, the number of sporangia per leaflet produced on leaflets taken from plants drenched with 0, 0.5, 5, and 10 mg a.i. of metalaxyl per plant was 139,600 ($\pm 44,000$), 219,600 ($\pm 68,000$), 192,800 ($\pm 60,000$), and 103,300 ($\pm 22,000$), respectively. Sporulation was stimulated (significant, $P = 0.05$) in leaflets detached from plants treated with 0.5 and 5 mg a.i. of metalaxyl per plant.

The isolate collected 27 April was relatively sensitive to metalaxyl (Table 1). To determine whether it could be adapted to tolerate higher metalaxyl concentrations (2,6), the fungus was successively propagated for three generations on plants treated in succession with 0.2, 0.5, and 1.25 mg a.i. of metalaxyl, one generation at each of the three doses. The sporangia produced in the third generation were as infective on plants drenched with 5 mg a.i. per plant and plants sprayed with 750 μg a.i./ml as to fungicide-free plants. The sporangia collected from the treated plants were highly infective (fifth generation) to plants drenched with 15 mg a.i. of metalaxyl per plant.

DISCUSSION

The data presented in this paper indicate that metalaxyl-resistant populations of *P. infestans* occur in Israel. Such populations apparently did not exist in the country until the spring of 1982, as all nine isolates collected during 1977–1981 were sensitive to the fungicide. Metalaxyl-mancozeb was labeled for commercial use in potatoes in Israel in 1981.

There are at least three (separate or combined) possible sources of the resistant populations in Nahal-Oz, our main site of sampling: in situ buildup due to selection pressure imposed by the fungicide; windborne inoculum imported from neighboring infected fields in Yad-Mordechai, located 15 km west of Nahal-Oz; and tuberborne inoculum imported from abroad. The first possibility is supported by the fact that the isolates of *P. infestans* collected on 27 April from a field sprayed with metalaxyl on 25 April and 9 May was relatively sensitive to metalaxyl, but those isolated at 2 and 4 wk later were highly resistant.

We have no conclusive evidence to support the second and third possibilities, but the fact that tubers of cultivar Cardinal were imported from Holland, where resistant populations of *P. infestans* occur (7), may support the third possibility. If correct, the imported resistant fungus increased and strengthened in Nahal-Oz with the aid of the metalaxyl used.

Further work is needed to establish whether the tolerance to metalaxyl in isolates from Israel is an expression of genetic constitution or adaptive physiology. Davidse (6) obtained isolates of *P. megasperma* f. sp. *medicaginis* that were resistant to metalaxyl by mycelial adaptation. Bruin and Edgington (2) reported on adaptation to metalaxyl in vitro in *Pythium* and *Phytophthora* species, but not in *P. infestans* and *Peronospora parasitica* in vivo. The low level of tolerance to metalaxyl of our 27 April isolate may have been increased in nature, as well as in the laboratory, either due to selection or physiological adaptation.

Noteworthy is the fact that in some experiments we observed that metalaxyl at some concentrations favored sporulation of the blight fungus. It remains to be studied whether metalaxyl-dependent types of *P. infestans* occur in nature.

Whatever was the source of the resistant population, our observation showed clearly that two sprays of metalaxyl-mancozeb, which we would expect to protect the plants against blight for at least 1 mo, were totally ineffective. Disease increased from 0.1 to 100 infected leaflets per plant during a period of 4 wk with an apparent infection rate of 0.26 per unit per day.

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