Metalaxyl-Resistant Strains of *Pseudoperonospora cubensis* in Cucumber Greenhouses of Southern Greece

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ABSTRACT

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No systemic or translaminar activity of metalaxyl against three strains of *Pseudoperonospora cubensis* could be demonstrated in artificially inoculated cucumber leaves, although the fungicide concentrations were several times higher than recommended. Some protective action was noticed but was unsatisfactory compared to that of a standard protectant. The strains appear to possess stable metalaxyl resistance.

The demand for fresh vegetables during the winter season has made the use of plastic houses for growing tomatoes and cucumbers very profitable in some areas, particularly in southern Greece. Because of the favorable climate, little or no expenditure is required for heating. This practice, however, creates conditions extremely favorable for some diseases that are difficult to control without the use of highly effective chemicals. The systemic fungicides, particularly metalaxyl, have been very useful for the control of Oomycetes in plastic houses in Greece. In the 1979–1980 growing season, however, growers from the island of Crete and one area in western Peloponnese reported failures in controlling cucumber downy mildew with metalaxyl in their greenhouses; their crops were almost totally destroyed.

We investigated the possibility of metalaxyl resistance in strains of the pathogen, *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow., from the problem areas. Similar work is being conducted in two other laboratories in Greece (3,4).

METHODS AND RESULTS

To obtain isolates of the fungus from the problem areas, we washed diseased material thoroughly with tap water to remove fungicide residues and old sporangia. Leaves were then incubated overnight in a moist chamber. Suspensions of fresh sporangia were obtained by agitating a small volume of distilled water containing small pieces of leaves bearing sporangiophores. Potted cucumber plants of the cultivar Herta, with four to eight true leaves, were inoculated by placing droplets of sporangial suspensions on the lower leaf surface. Each droplet (about 0.015 ml) contained approximately 150 sporangia. The plants were then covered with wet plastic bags for 48 hr. Depending on the condition of the leaf, this method of inoculation was often 100% successful. Chlorotic spots appeared within 4–5 days at about 25 C. The isolates were maintained by transferring sporangia to new untreated plants every 7–10 days.

The sensitivity of the fungus to metalaxyl was determined by counting the number of spots produced after artificial inoculation of treated and untreated leaves. Ridomil 25 WP was the metalaxyl formulation used. Sprays were applied until runoff with a small hand sprayer. For the soil drench, 20 ml of an appropriate preparation was added to the soil in each 12-cm-diameter pot. The controls were similarly sprayed or drenched with tap water.

Three types of metalaxyl treatment were tested. In the first type, the upper surface of each leaf was carefully sprayed with metalaxyl at 250 (the recommended dose), 500, or 1,000 μg/ml; droplets with sporangia were placed on the lower surface 1 or 24 hr later. With three isolates of *P. cubensis* from problem greenhouses (two from Crete, one from western Peloponnese), the spray treatments had no effect on the disease. Even on plants that were sprayed with the highest fungicide concentration, almost every droplet of inoculum produced a lesion that was indistinguishable from the lesions on untreated plants (Fig. 1). Sporulation was not affected by these treatments. The higher metalaxyl concentrations were sometimes phytotoxic.

A soil drench was the second type of metalaxyl treatment. Metalaxyl was applied at 0.20, 0.40, 0.75, and 1.5 g/m^2 (0.10 g/m^2 is recommended). These soil drenches also had no effect on downy mildew. The lack of control was not due to absence of fungitoxic material from the leaves of the treated plants. When small pieces of leaf blade were surface-sterilized and placed on potato-dextrose agar, strong inhibition of a *Pythium* sp. was observed in leaves from the root treatment, but no inhibition occurred in the control (Fig. 2).

These two types of experiments clearly showed that metalaxyl did not exert systemic control of the three isolates of *P. cubensis*.

We examined the possibility of protective action by carefully applying metalaxyl at 250 or 500 μg/ml to the lower leaf surface; after the leaves were dry, the droplet inoculations were made. This treatment tended to reduce the

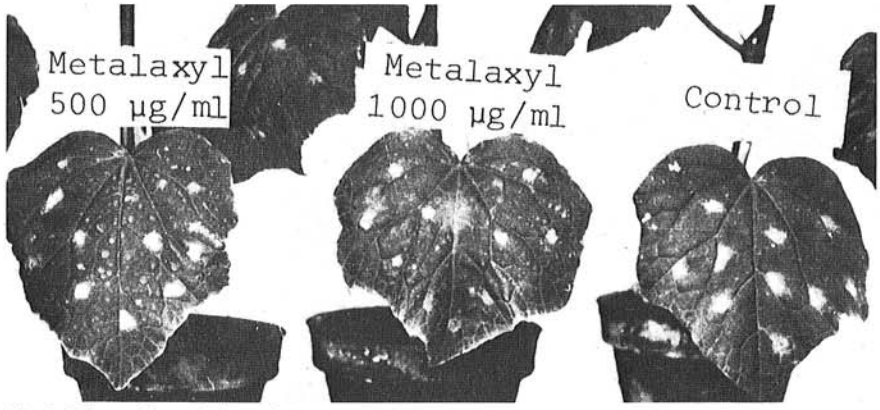

Fig. 1. Effect of inoculations of the lower surface of cucumber leaves with a suspension of sporangia of a metalaxyl-resistant strain of *Pseudoperonospora cubensis*. The upper surface of the leaves was sprayed 1 hr before inoculation with the metalaxyl or with tap water (control).

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number of spots produced, but protection was very poor compared to that obtained with propineb (Antracol 65), which did not allow any symptoms to appear. The spots that developed after metalaxyl treatment of the lower leaf surface were indistinguishable from spots on untreated plants. Sporulation was abundant, and when used in artificial inoculations, sporangia from treated leaves behaved normally. The number of spots was not reduced when the fungicide was sprayed on the lower surface of the leaf 48 hr after inoculation, indicating complete lack of therapeutic activity.

The slight protection of the leaf was even less when the same concentrations of recrystallized metalaxyl, instead of the commercial product, were applied on the lower surface. This seems to indicate some role in protection by other components of the commercial preparation. We observed that droplets containing sporangia often tended to spread when placed on surfaces sprayed with the commercially prepared metalaxyl. This might create an environment less favorable for infection. Even with the recrystallized metalaxyl, however, protective activity was obvious at high concentration.

Table 1. Effect of fungicide treatments on cucumber downy mildew after artificial inoculation with a metalaxyl-resistant isolate of *Pseudoperonospora cubensis*

Fungicide	Application	Total number of	
		Inoculations	Lesions
None		330	311
Metalaxyl	Soil drench (g a.i./m^2)		
(commercial)	0.20	70	61
	0.40	30	28
	0.75	40	40
	1.50	100	89
	Spray (μg a.i./ml)		
	Upper leaf surface		
	250	180	167
	500	260	257
	1,000	50	50
	Lower leaf surface		
	250	190	100
	500	120	32
Metalaxyl	Lower leaf surface		
(recrystallized)	250	70	56
	500	80	46
Propineb	Spray (μg a.i./ml)	80	0
	Lower leaf surface		
	1,300		

The results of the experiments conducted with one of our isolates are summarized in Table 1. This isolate was maintained by artificial inoculation of untreated plants for 20 generations over 7 mo. Each experiment used inoculum from recently infected untreated leaves, and the metalaxyl sensitivity of the isolates did not differ among experiments conducted at various times during the 7 mo.

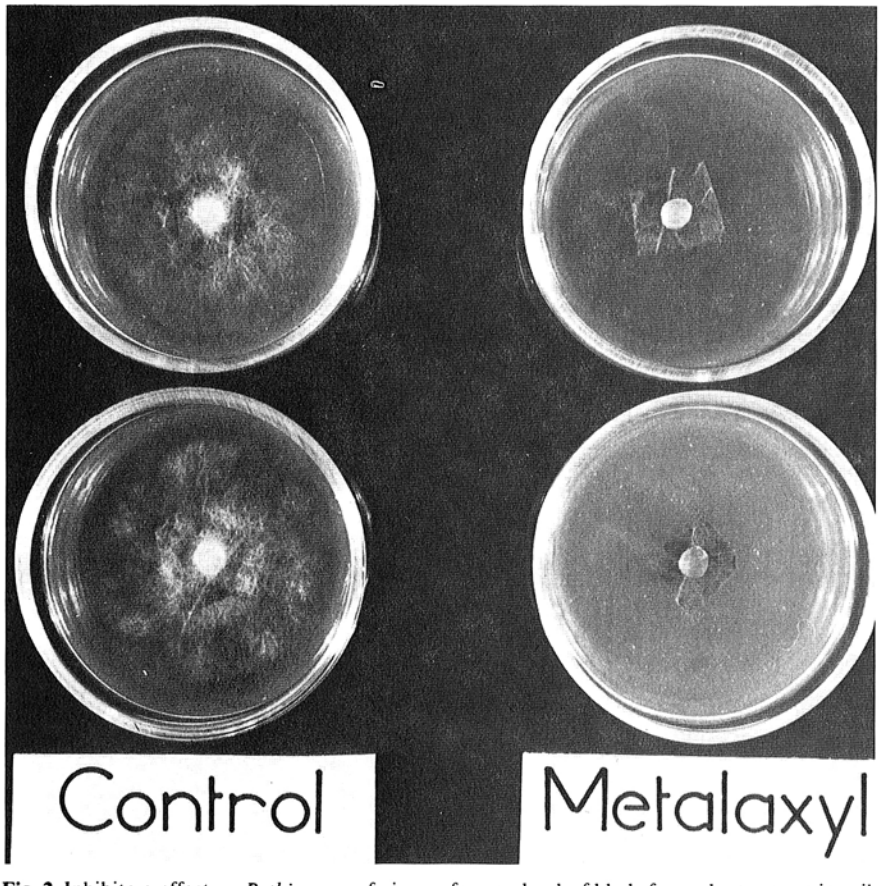

Fig. 2. Inhibitory effect on *Pythium* sp. of pieces of cucumber leaf blade from plants grown in soil treated with metalaxyl (1.5 g/m^2). The pieces were cut 24 hr after the treatment, surface sterilized, and placed on potato-dextrose agar. Then a block (5 mm diameter) from a culture of *Pythium* sp. was placed on each leaf piece.

DISCUSSION

Strains of the downy mildew pathogen, *P. cubensis*, which cannot be controlled by metalaxyl, are present in cucumber greenhouses of southern Greece. Three isolates from such greenhouses are highly pathogenic and are capable of normal growth and reproduction on plants subjected to metalaxyl foliar sprays or soil drenches at concentrations exceeding those recommended by the manufacturer. There can be little doubt that such strains of the pathogen can be responsible for failures of the fungicide in commercial operations. Involvement of metalaxyl-resistant strains in failures of control of the downy mildew of cucurbits has also been noticed in Israel (T. Katan and E. Bashi, *personal communication*).

In our experiments, metalaxyl applied to the lower leaf surface on which the sporangia were placed partially inhibited the development of strains that were completely unaffected by the fungicide applied on the opposite leaf surface or through the roots (Table 1). In view of the systemic nature of the compound, this difference cannot easily be explained. Metalaxyl treatment of the lower leaf surface may permit higher concentrations inside the leaf, perhaps due to the presence of a greater number of stomata. This, however, would not explain why the fungicide does not have a similar effect when applied on the lower surface of the leaf after infection. Higher sensitivity of the fungus (eg, its zoospores) before infection would, of course, be another possibility. In the case of *Plasmopara viticola* (Berk. & Curt.) Berl. & deT., and *Phytophthora infestans* (Mont.) de By., however, metalaxyl is not effective until after initiation of the first haustorium (5). Whether this is true also in the case of *P. cubensis* is not known.

Although our isolates were not directly compared with an unquestionably wild type strain of *P. cubensis*, the baseline metalaxyl sensitivity of this species must be considerably higher than what we observed for the following reasons: 1) In field experiments by the manufacturer before introduction of the fungicide, foliar sprays with metalaxyl at 280 and 500 μg/ml achieved fair and perfect control, respectively, of *P. cubensis* on squash (6). Growers and advisory personnel in southern Greece also reported very good performance of metalaxyl during the 1978–1979 growing season. 2) Strains of *P. cubensis* unable to infect metalaxyl-treated cucumber plants have been tested in Israel (T. Katan and E. Bashi, *personal communication*) and in Greece (3,4).

It can be concluded that our isolates possess stable and apparently genetically controlled resistance to metalaxyl. Whether resistant strains exist, even before the fungicide is used in an area, and in what frequency is not known. Where resistant strains are present in the pathogen population, their frequency will be increased by metalaxyl treatments, with resultant failure in disease control. In the laboratory, strains of other Oomycetes with stable metalaxyl resistance and also pathogenic on their respective hosts have been obtained (1,2). So far in nature, however, such strains have been found only in *P. cubensis*.

Unusually high selection pressure probably was applied to the population of this pathogen by repeated metalaxyl applications in the cucumber greenhouses during the entire 1978–1979 growing season. Although the likelihood of metalaxyl resistance developing in other plant pathogenic Oomycetes may be considerably lower, this possibility must be considered particularly in the case of diseases of major importance.

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