Resistance to Metalaxyl in Isolates of *Pseudoperonospora cubensis*, the Downy Mildew Pathogen of Cucurbits

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ABSTRACT

Katan, T., and Bashi, E. 1981. Resistance to metalaxyl in isolates of *Pseudoperonospora cubensis*, the downy mildew pathogen of cucurbits. Plant Disease 65:798-800.

Metalaxyl-resistant *Pseudoperonospora cubensis* was isolated from infected cucumber leaves in a greenhouse in Israel where metalaxyl failed to control downy mildew. The resistant isolate caused typical infections on cucumber plants sprayed with metalaxyl (up to 500 μg a.i./ml) 2 days before or 2 days after inoculation. Disease symptoms and severity on sprayed plants were similar to those on unsprayed inoculated control plants. The resistance was stable through 10 successive inoculations in the absence of metalaxyl and did not affect pathogenicity.

Additional key words: acylalanine fungicides, tolerance

When first introduced in the winter of 1978–1979, metalaxyl provided a high level of control of downy mildew, caused by *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow, on cucumbers (*Cucumis sativus* L.) in Israel. The recommended rate of metalaxyl was 250 g a.i./ha at 3-wk intervals as preventive sprays and at 10- to 14-day intervals to control low levels of disease. For control of epidemics, two sprays within 5 days were recommended, followed by further sprays at 7- to 10-day intervals according to disease severity. In November 1979, however, a severe outbreak of downy mildew on greenhouse-grown winter cucumbers regularly sprayed with metalaxyl was reported from the Hadera region in central Israel. The disease could not be controlled even by increasing the frequency of metalaxyl sprays, and protectant fungicides had to be used.

The present research was undertaken to determine if the failure to control downy mildew was because resistance to metalaxyl had developed in *P. cubensis*.

MATERIALS AND METHODS

The host. Cucumber (*C. sativus* 'Delila') plants were grown in the greenhouse in 0.3-kg pots containing sand, soil, and peat (1:1:1). The experiments were performed with plants at the two or three true leaf stage.

The pathogen. The wild type, metalaxyl-sensitive isolate BI/S of *P. cubensis* has been maintained in the laboratory since 1976, before metalaxyl was introduced as an experimental fungicide. Isolate NA/R, suspected of being resistant to metalaxyl, was isolated by inoculating cucumber seedlings with sporangia from infected leaves of winter-grown cucumbers obtained in April 1980 from a greenhouse where metalaxyl failed to control downy mildew.

In the summer (July 1980), two additional isolates, ME-1 and CU-1, were isolated from infected leaves of melon (*C. melo* L.) and cucumber grown in open fields, respectively. The melon isolate originated from Hadera region, where we had recorded the failure of metalaxyl to control downy mildew on cucumbers the preceding winter. The cucumber isolate originated from an experimental plot in which metalaxyl had been used regularly.

Each isolate was maintained and propagated by weekly inoculation on cotyledons of cucumber seedlings. Formation of sporangia was induced by placing the infected seedlings, after 8 hr in the light, in a 20 C humidity chamber in darkness, overnight (3). Sporangia were brushed from the cotyledons into deionized water by using a soft-hair brush.

Fungicide application and inoculation. Metalaxyl (Ridomil 25W) was prepared as aqueous suspensions of varying concentration. Two series of experiments were performed employing two methods for applying metalaxyl to the plants and for inoculating plants with *P. cubensis*.

In the target method, a 21-cm^2 area in the center of the upper (adaxial) side of each leaf was sprayed with a fixed volume (about 0.01 ml) of metalaxyl suspension, by using a modified Schein inoculator (1). For quantitative inoculation, a sporangial suspension (1.2–1.4 × 10^4 sporangia per milliliter) was directed with the inoculator at a 3.8-cm^2 area in the center of the lower (abaxial) side of each leaf. Glass slides, sprayed with the sporangial suspension in the same manner as for leaf inoculation, were used to estimate the number of sporangia deposited on the leaves; the average was 160 ± 30 sporangia per target area. For comparison, the protectant fungicide mancozeb (Dithane M-45W) was used. The entire upper surface of each leaf was sprayed with an aqueous

Table 1. Effect of metalaxyl and mancozeb on infection of cucumber plants by three isolates of *Pseudoperonospora cubensis*

Fungicide (μg a.i./ml)	Isolate BI/S[a] Infected leaves (%)	BI/S[a] Disease index[d]	NA/R[b] Infected leaves (%)	NA/R[b] Disease index	ME-1[c] Infected leaves (%)	ME-1[c] Disease index
Unsprayed control	86	B–C/3	100	B–C/5	100	C/4
Metalaxyl 25	0	···	100	C/5	100	C/5
125	0	···	86	B–C/5	100	B–C/5
Mancozeb 2,000	0	···	0	···	NT[e]	···

[a] Metalaxyl sensitive, wild type—14 leaves per treatment.
[b] Metalaxyl resistant, greenhouse—14 leaves per treatment.
[c] Metalaxyl resistant, open field—22 leaves per treatment.
[d] Lesion color: A (chlorotic) to C (necrotic). Infection grade: 1 (few spots) to 6 (total coverage of inoculated target).
[e] NT, not tested.

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suspension (2,000 μg a.i./ml), and 2 days later the leaves were inoculated quantitatively on the sprayed side. Seven plants were used per treatment.

In the cover-all method, metalaxyl was freely sprayed over the entire foliar surface of the plants, by using a household hand-sprayer. The leaves were inoculated with a sporangial suspension (about 10^4 sporangia per milliliter) by using a DeVilbiss atomizer operated by hand and aimed to cover the upper surface of the leaves. Three plants were used in this treatment.

Inoculated plants were kept in humidity chambers at 20 C in the dark for 20 hr and then transferred to a growth chamber at 20 C with a 12-hr photoperiod. Disease severity was estimated 6 days after inoculation. In the target method the disease index was based on: lesion color, which was graded A (chlorosis) to C (necrosis); and on the relative area of the target covered by infection, which was graded 1 (few spots) to 6 (total coverage), as described by Cohen and Rotem (4). Plants inoculated by the cover-all method were evaluated after 6 days as infected or uninfected.

RESULTS

With the target method, the sensitive isolate BI/S failed to infect plants that had been sprayed with metalaxyl at 25 μg a.i./ml 2 days before inoculation but caused infection of 30–50% of plants that had been sprayed 5 days before inoculation. Table 1 summarizes the results obtained from inoculations with isolates BI/S, NA/R, and ME-1. No infection by isolate BI/S was found on plants that had been sprayed with metalaxyl at 125 or 250 μg a.i./ml 2 or 5 days before inoculation; unsprayed plants became infected. Isolate NA/R, suspected of being resistant to metalaxyl, and isolates ME-1 and CU-1 caused typical infections when inoculated onto plants that had been sprayed 2 days earlier with metalaxyl at 25–250 μg a.i./ml. Isolates BI/S and NA/R failed to infect plants that had been sprayed with mancozeb 2 days before inoculation.

By the cover-all method, no infection was caused by the sensitive isolate BI/S on plants sprayed with metalaxyl at 125 μg a.i./ml 2 days before or 2 days after inoculation; unsprayed plants became infected (Fig. 1A). On the other hand, spraying the plants with metalaxyl at concentrations of 125–500 μg a.i./ml 2 days before or 2 days after inoculation by isolate NA/R did not prevent or delay the infection (Fig. 1B), indicating that this isolate was resistant to the fungicide. Metalaxyl at 0.2% formulated compound, the equivalent of 500 μg a.i./ml, was often phytotoxic and caused light burns at leaf margins. This did not affect development of symptoms caused by isolate NA/R.

Metalaxyl resistance of isolate NA/R persisted through more than 10 successive inoculations on cucumber seedlings in the absence of metalaxyl. In either experimental method, downy mildew symptoms and disease severity on metalaxyl-treated cucumber plants were similar to those on unsprayed, inoculated control plants. Sporangia of isolate NA/R that were induced on metalaxyl-treated plants were infective to sprayed and to unsprayed plants.

DISCUSSION

These results confirmed the suspicion that failure to control downy mildew by metalaxyl could be attributed to the development of resistance to this fungicide in *P. cubensis*. Metalaxyl resistance of isolate NA/R persisted in the absence of metalaxyl and did not reduce its pathogenicity. Resistant *P. cubensis*, which first appeared on winter-grown cucumbers, was found in the same region on summer-grown melons several months after use of metalaxyl had been halted, indicating that resistant strains could survive in the absence of metalaxyl and pass from one season to another and from greenhouses to the open field.

Induction of metalaxyl-resistant types or their selection from natural populations has been demonstrated in *Phytophthora* spp. and *Pythium* spp. (2,5,9). Staub et al (9) pointed to the necessity for field evaluation of the practical risk of resistant types selected in vitro. Artificially induced isolates of *Pythium* maintain their partial resistance in the field but can be controlled by 10 times the normal amount of metalaxyl (2); seedling damping-off caused by resistant isolates

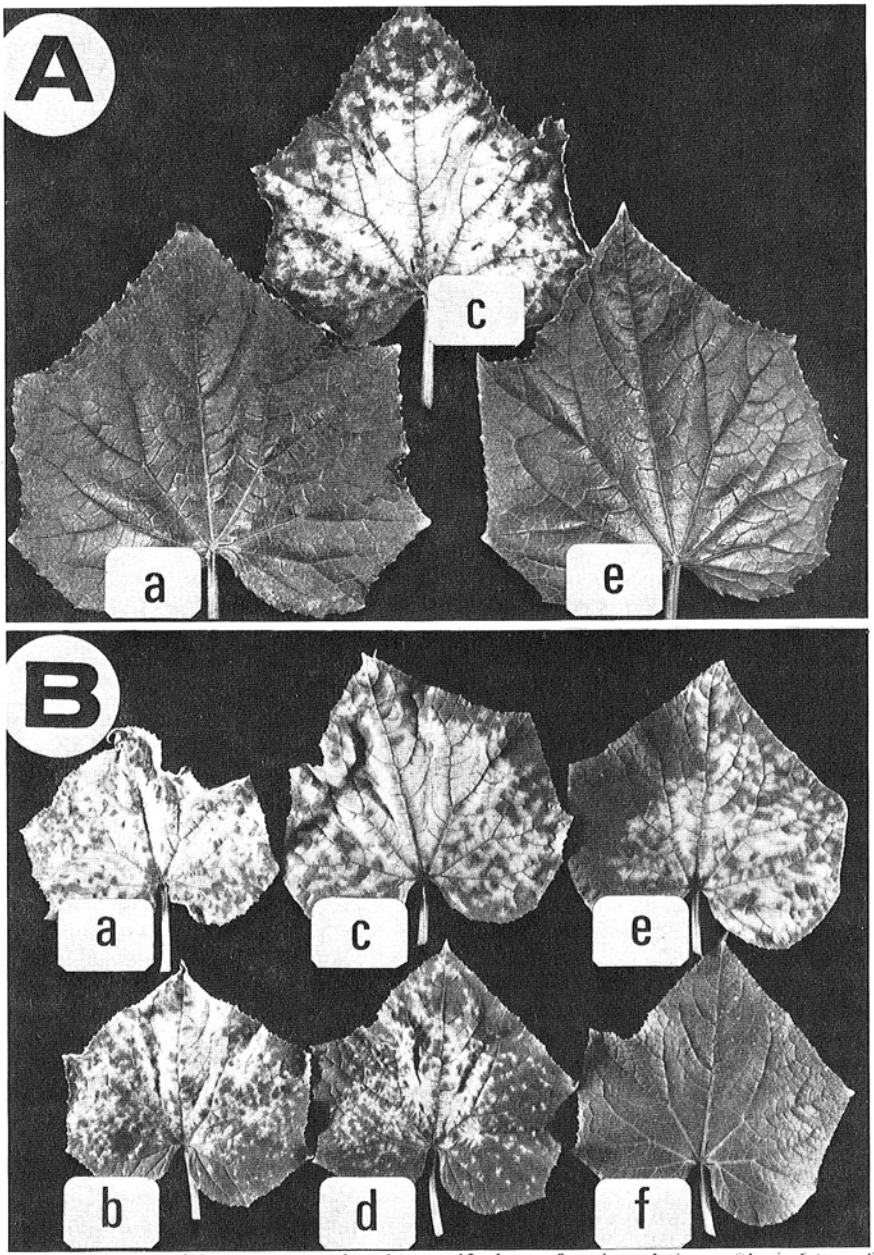

Fig. 1. Downy mildew on cucumber leaves 10 days after inoculation with isolates of *Pseudoperonospora cubensis* **(A)** sensitive or **(B)** resistant to metalaxyl. Plants were sprayed with metalaxyl at **(a)** 125, **(b)** 250, or **(d)** 500 μg/ml, with **(f)** mancozeb at 2,000 μg/ml 2 days before inoculation, or with **(e)** metalaxyl at 125 μg/ml 2 days after inoculation; **(c)**, unsprayed control.

of *P. megasperma* (5) are not controlled by high concentrations of metalaxyl. In our study, metalaxyl-resistant *P. cubensis* could not be controlled by increasing the fungicide concentration to a phytotoxic level. Similarly, reducing the intervals between metalaxyl sprays did not solve the problem in commercial greenhouses, forcing the growers to return to the previously used, less effective protectant fungicides.

Our study shows that strains of the cucurbit downy mildew fungus that are resistant to metalaxyl may emerge after intensive use of this fungicide. Recently, a similar phenomenon was reported from Crete and Greece (6–8). Resistance appears to be stable. Although the cucumber-downy mildew pathosystem is not highly important to global economy, it should be considered when metalaxyl is incorporated into chemical control programs of economically important diseases such as tobacco and grape downy mildews and potato late blight.

Added in galley: After this article was accepted, resistance to metalaxyl was reported in *Phytophthora infestans* (Davidse et al, Neth. J. Pl. Pathol. 87:65-68) and in *Peronospora tabacina* and *Plasmopara viticola* (Staub and Sozzi, Neth. J. Pl. Pathol., in press).

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