Development of Resistance to Metalaxyl in *Pseudoperonospora cubensis*

M. REUVENI, H. EYAL, and Y. COHEN, Department of Life Sciences, Bar-Ilan University, Ramat Gan, Israel

ABSTRACT

REUVENI, M., EYAL, H., and COHEN, Y. 1980. Development of resistance to metalaxyl in *Pseudoperonospora cubensis*. Plant Disease 64:1108-1109.

A metalaxyl-resistant strain of *Pseudoperonospora cubensis* was isolated in December 1979 from cucumber plants growing in plastic houses on the northern coastal plain of Israel. Plants at the two-leaf stage, drenched with 15 mg (a.i.) of metalaxyl per 0.5-L pot or sprayed with 500 μg per leaf, became heavily mildewed when inoculated with the resistant strain, but 50 μg/0.5-L pot or 25 μg per leaf effectively controlled the disease in plants inoculated with the sensitive strain. Both strains of the pathogen had the same physiologic specialization when introduced on six members of the Cucurbitaceae.

Metalaxyl (Ridomil) is a systemic fungicide specifically active against Peronosporaceous fungi (7). It is highly effective for controlling downy mildews, having both protective and curative properties (7; Y. Cohen, *unpublished*). Unlike the dithiocarbamates and other protectant fungicides that block spore germination, metalaxyl has little effect on germination but does greatly reduce fungal growth in both culture (7) and plant tissue (2). Postinfection application of the chemical 48 hr after inoculation of tomato leaves with *Phytophthora infestans* (Mont.) de Bary greatly reduced disease development (2). Foliar spray, soil drench, or seed infiltration are all useful techniques for applying the compound. A single soil treatment of potted tomato plants effectively controlled the late blight disease after 65 days (2).

Numerous examples of development of resistance to fungicides in populations of plant pathogenic fungi have been reported (3,4). Resistance to systemic fungicides, such as benomyl, was recorded before they were in commercial use (5). Staub et al (6) showed that prolonged exposure of *P. infestans* to sublethal dosages of metalaxyl on agar or on potato plants failed to produce virulent strains with resistance to the chemical.

The present study is the first report that a metalaxyl-resistant strain of the obligate fungal parasite *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow, the incitant of downy mildew in cucurbits, has developed in Israel.

In preliminary studies, we noted that heavy epiphytotics of downy mildew developed on cucumbers sprayed with metalaxyl and grown in plastic houses in

0191-2917/80/12110802/$03.00/0

Table 1. Disease intensity in cucumber plants treated with metalaxyl and inoculated with *Pseudoperonospora cubensis*

Metalaxyl treatment	Disease intensity[a] in plants inoculated with strain	
	MS[b]	MR[c]
μg a.i./0.5-L pot		
0	3.4 ± 0.6	5.0 ± 0
50	0.4[d] ± 0.2	4.9 ± 0.2
250	0.4[d] ± 0.1	5.0 ± 0
500	0.4[d] ± 0	4.4 ± 0.5
2,500	0.3[d] ± 0.1	4.8[e] ± 0.3
5,000	...	4.9[e] ± 0.2
10,000	...	4.7[e] ± 0.5
15,000	...	4.8[e] ± 0.5
μg a.i./leaf		
0	3.4 ± 0.6	5.0 ± 0
25	0.4[d] ± 0.4	4.4 ± 0.6
125	0.3[d] ± 0.2	4.1 ± 0.8
250	0.3[d] ± 0.2	3.1 ± 0.5
500	...	3.8[e] ± 1.0
750	...	0.2[e] ± 0.2

[a] 0–5 visual scale (% target area infected × color index, see [1]) ± standard deviation of the mean (n = 16).
[b] MR = wild type metalaxyl-sensitive.
[c] MS = metalaxyl-resistant.
[d] Minute, sterile lesions.
[e] Phytoxicity, leaf margins "burn."

the Hadera district during the winter of 1979-1980. As many as 16 sprays during the season did not control the disease. Mancozeb used similarly in neighboring plastic houses controlled the disease efficiently. In some plastic houses in which cucumbers were treated with metalaxyl, total yield loss occurred. Greenhouse-grown potted cucumbers that were treated with metalaxyl and placed in these plastic houses for 10 days became heavily mildewed. When inoculated in the laboratory with the wild-type strain, such plants were completely free of disease.

MATERIALS AND METHODS

All experiments were done with the susceptible cucumber (*Cucumis sativus* L. 'Bet-Alpha'). Metalaxyl was applied as a soil drench or foliar spray to two two-leaf plants in 0.5-L pots. Soil was drenched with 20 ml of 25% WP formulation of metalaxyl at 50, 250, 500, 5,000, 10,000, and 15,000 μg a.i. per pot. Foliage was sprayed with 1 ml of metalaxyl suspension per leaf on both leaf surfaces at 25, 125, 250, 500, and 750 μg a.i. per leaf.

Three hours after metalaxyl was applied, the plants were inoculated with *P. cubensis* on 4.5 cm^2 of the lower leaf surface with about 100 ± 20 sporangia per square centimeter, using a Schein apparatus, then placed in moist chambers in the dark at 15 C. After 20 hr, the plants were transferred to a growth cabinet (20 C, 12-hr photoperiod, light intensity of 150 μE $m^{-2}s^{-1}$) for lesion production.

Two strains of the pathogen were used for inoculation. A wild type, designated as metalaxyl-sensitive (MS), was collected in June 1976 from an open cucumber field in the Sharon Plain of Israel and maintained on cucumber plants by repeated inoculation in growth chambers. The second strain was isolated from metalaxyl-treated cucumber plants growing in plastic houses in the Hadera district in December 1979 and designated as metalaxyl-resistant (MR). Care was taken to keep the two strains separate. Seven days after inoculation, disease was rated on a visual 0–5 scale (1). Plants were then kept in moist chambers in the dark at 20 C for 20 hr to allow fungal sporulation.

Other cucurbits also were inoculated with each strain to observe whether host-range changes had occurred (Y. Cohen, *unpublished*).

RESULTS

Microscopic examination of the two strains of the pathogen revealed no morphological differences in size, shape, or pigmentation of sporangia or sporangiophores. In both strains, zoospore release was equally insensitive to metalaxyl up to 125 μg a.i./ml, but mancozeb at about 1 μg/ml caused a 50% reduction in zoospore release.

Low doses of metalaxyl effectively controlled the disease incited by the MS strain (Table 1). Plants treated with ≥ 50 μg per pot or ≥ 25 μg per leaf when inoculated with MS strain produced small (0.5–1 mm diameter) chlorotic lesions that produced no sporangia when the leaves were placed at 100% RH. However, metalaxyl did not control the disease incited by the MR strain, except at the highest dose of 750 μg per leaf in the spray treatment, nor did metalaxyl affect the sporulation or spore viability of the pathogen on any lesions that developed.

Sporangia collected from metalaxyl-treated plants inoculated with the MR culture maintained their metalaxyl-resistant characteristic even when passed for six generations through untreated plants. The possibility that the MR strain was a *forma specialis* different from the MS strain was tested by comparative inoculations with the two strains of *Cucumis melo* L. (PI 142111), *Lagenaria vulgaris* Ser., *Luffa cylindrica* (L.) Roem, *Citrullus lanatus* Schard., *Cucurbita moschata* Poir., cv. Dickenson pumpkin, and *Cucurbita maxima* Duch. Typical resistant reactions were observed on all species regardless of the strain used.

DISCUSSION

The natural distribution and frequency of MR strains of *P. cubensis* in Israel has not yet been determined, but a national survey is under way. A resistant strain was isolated in an area where metalaxyl has been used on cucumbers for 3 yr. Continued commercial use of this chemical may cause the problem of resistant strains to become acute.

P. cubensis is not known to reproduce sexually in cucurbits in Israel. The evolution of a resistant strain may be attributed to selection by the fungicide from the natural population. The resistant strain that sporulated abundantly on leaves may have predominated in plastic houses because the fungicide eliminated the sensitive fungal population (3). It is possible, however, that the chemical induced a genetic recombination in the pathogen (3,4). Limited host range experiments indicate that the new strain has a physiological specialization similar to that of the wild type strain.

The breakdown of this newly developed fungicide poses the serious threat of causing evolution of resistant strains in sexually reproducing Phycomycetes, such as *Peronospora tabacina* Adam, *Peronosclerospora sorghi* (Weston & Uppal) Shaw, *Phytophthora* spp., and *Pythium* spp.

ACKNOWLEDGMENTS

We are grateful for the assistance of A. Cohen, Department of Life Sciences, Bar-Ilan University, and Y. Sachs of the Ministry of Agriculture.

LITERATURE CITED

1. COHEN, Y. 1977. The combined effects of temperature, leaf wetness, and inoculum concentration on infection of cucumbers with *Pseudoperonospora cubensis*. Can. J. Bot. 55:1478-1487.
2. COHEN, Y., M. REUVENI, and H. EYAL. 1979. The systemic antifungal activity of Ridomil against *Phytophthora infestans* on tomato plants. Phytopathology 69:645-649.
3. DEKKER, J. 1976. Acquired resistance to fungicides. Annu. Rev. Phytopathol. 14:405-428.
4. HASTIE, A. C. 1979. Genetic effects of fungicides. 9th Int. Congr. Plant Prot., Washington, DC, 1979. 351 pp.
5. SCHROEDER, W. T., and R. PROVVIDENTI. 1969. Resistance to benomyl in powdery mildew of cucurbit. Plant Dis. Rep. 53:271-277.
6. STAUB, T., J. DAHMEN, P. URECH, and F. SCHWINN. 1979. Failure to select for in vivo resistance in *Phytophthora infestans* to acylalanine fungicides. Plant Dis. Rep. 63:385-389.
7. URECH, P. A., F. SCHWINN, and T. STAUB. 1977. CGA 48988, a novel fungicide for the control of late blight, downy mildews and related soil-borne disease. Pages 623-631 in: Br. Crop Prot. Conf. Proc.