Differential Sensitivity to Mancozeb of Metalaxyl-Sensitive and Metalaxyl-Resistant Isolates of *Pseudoperonospora cubensis*

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ABSTRACT

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Eight field isolates of *Pseudoperonospora cubensis*, the fungal causal agent of downy mildew in cucurbits, were tested for sensitivity to metalaxyl and mancozeb. While mancozeb efficiently controlled downy mildew in cucumbers inoculated with three metalaxyl-sensitive isolates, it failed to do so in cucumbers inoculated with five other metalaxyl-resistant isolates. The practical and theoretical implications of this finding are discussed.

Additional key words: chemical control strategy, cross-resistance, protectant fungicides, systemic fungicides.

Reduced disease control caused by resistance to a chemical agent in farming practice usually prompts a change to another chemical agent. To combat resistance to systemic (site-specific) fungicides, mixtures or alternating sprays with protectant (non-site-specific) fungicides are suggested (3,4,8). The idea behind these practices is that protectant fungicides, being toxic at multiple sites in the cell, are expected to eliminate subpopulations both sensitive and resistant to a site-specific fungicide.

In a recent paper (1), we demonstrated that isolates of *Pseudoperonospora cubensis* resistant to metalaxyl exhibited cross-resistance to four other systemic fungicides: vinicur, SAN 371F, propamocarb, and Aliette. The present study was undertaken to determine the control efficacy of the protectant fungicide mancozeb against downy mildew in cucumbers inoculated with sensitive and resistant isolates of *P. cubensis*.

MATERIALS AND METHODS

Three metalaxyl-sensitive (MS) and five metalaxyl-resistant (MR) isolates of *P. cubensis*, collected in Israel, were used in this study (Table 1). Isolates were chosen from a collection of 23 isolates so as to include various degrees of sensitivity to metalaxyl. Fungicide-use histories in the areas from which isolates were collected were not available except for MS2 (regular sprays with mancozeb) and MR1 (frequent sprays with metalaxyl [6]). The MS fungal isolates were propagated on fungicide-free cucumber plants while the MR isolates were propagated on metalaxyl-treated cucumber plants in separate growth chambers with strict measures undertaken to avoid cross-contamination. MS isolates were simultaneously inoculated onto metalaxyl-treated plants to test their sensitivity to the fungicide.

The highly susceptible cucumber (*Cucumis sativum* L. 'Elem') was used in all inoculation experiments. Plants were grown in the greenhouse (20–32 C) in 10-cm-diameter plastic pots containing a mixture of sandy loam, vermiculite, and peat (1:1:1, v/v). Plants were used at either the cotyledonary leaf stage (about 10 days after sowing, 15 plants per pot) or the two-true-leaf stage (about 3 wk after sowing, one plant per pot). Plants were sprayed with either mancozeb (Dithane M-45 80WP) or metalaxyl (25WP) to initial runoff with the aid of a fine-mist homemade glass atomizer. Spray droplets were allowed to dry for about 2 hr and the plants were inoculated with a sporangial suspension of *P. cubensis*.

Cotyledonary leaves were inoculated by placing one 10-μl inoculum droplet (containing 5 ± 1 sporangia per droplet) on the adaxial surface of each cotyledon. Plants at the two-true-leaf stage were inoculated by spraying the adaxial leaf surfaces with a sporangial suspension (5,000 sporangia per milliliter) with a garden hand sprayer. Inoculated plants were placed in a dew chamber (Percival, I-60D) at 18 ± 1 C in the dark for about 20 hr, then transferred to a growth cabinet at 25 C (12 hr of light per day, about 150 μEinsteins $\cdot$ $m^{-2} \cdot s^{-1}$ supplied by VHO fluorescent lamps, 50–60% RH) for symptom production.

Disease data were recorded 7 days after inoculation. Where cotyledonary leaves had been inoculated, the number of infected leaves was counted. Where plants at the two-true-leaf stage had been inoculated, leaf area showing mildew symptoms on both inoculated leaves was measured by tracing on a transparent paper

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TABLE 1. Identification of eight field isolates of *Pseudoperonospora cubensis* used to study the differential sensitivity of metalaxyl-sensitive (MS) and metalaxyl-resistant (MR) isolates to mancozeb

Isolate	Collection date	Location	Host
MS1	July 1983	Kadima	Cucumbers, field
MS2	October 1983	Tira	Cucumbers, field
MS3	October 1983	Tul-Karem	Cucumbers, field
MR1	December 1979 (6)	Hadera	Cucumbers, plastic house
MR2	June 1983	Hadasim	Cantaloupe, field
MR3	August 1983	Kefar-Shemariyahu	Cucumbers, field
MR4	May 1983	Nir-Galim	Cantaloupe, field
MR5	October 1983	Neve-Yamin	Cucumbers, field

TABLE 2. Infectivity of three metalaxyl-sensitive (MS) and five metalaxyl-resistant (MR) isolates of *Pseudoperonospora cubensis* to cucumber cotyledonary leaves treated[a] with various concentrations of either metalaxyl or mancozeb

Fungicide	Fungicide conc. (μg a.i./ml)	Cotyledons infected (% ± SD)							
		MS1	MS2	MS3	MR1	MR2	MR3	MR4	MR5
Metalaxyl	0	100	100	100	100	100	100	100	100
	3.12	13.9 ± 5	5.0 ± 3	53.3 ± 6	···	···	···	···	···
	6.25	0	0	42.4 ± 5	···	···	···	···	···
	12.50	0	0	35.2 ± 6	···	···	···	···	···
	25.00	0	0	27.8 ± 10	···	···	···	···	···
	31.25	0	0	24.1 ± 5	98.7 ± 2	99.0 ± 1	86.1 ± 5	98.0 ± 2	100
	62.50	0	0	0	95.6 ± 3	98.1 ± 2	71.3 ± 9	90.0 ± 4	96.7 ± 7
	125.0	···	···	···	87.4 ± 8	94.1 ± 7	62.4 ± 6	93.3 ± 8	89.4 ± 8
	250.0	···	···	···	80.0 ± 6	91.3 ± 4	44.6 ± 8	84.8 ± 6	88.7 ± 8
	500.0	···	···	···	67.2 ± 8	84.2 ± 8	22.4 ± 2	80.1 ± 6	83.7 ± 5
	750.0	···	···	···	12.5 ± 5	14.8 ± 4	3.2 ± 5	7.6 ± 8	7.2 ± 6
Mancozeb									
	2.0	69.5 ± 7	8.1 ± 4	82.5 ± 11	99.8 ± 1	100	97.7 ± 5	98.7 ± 4	100
	4.0	53.6 ± 2	2.5 ± 4	66.3 ± 8	97.2 ± 5	98.4 ± 2	85.6 ± 8	96.9 ± 4	99.0 ± 1
	8.0	11.0 ± 4	0	22.5 ± 7	···	···	···	···	···
	16.0	0	0	0	88.1 ± 8	95.4 ± 6	78.2 ± 3	85.8 ± 9	96.0 ± 6
	24.0	0	0	0	62.9 ± 11	85.0 ± 8	52.3 ± 10	55.7 ± 5	76.5 ± 7
	32.0	···	···	···	45.2 ± 6	53.1 ± 3	13.1 ± 9	21.4 ± 5	44.8 ± 7
	40.0	···	···	···	24.4 ± 7	41.2 ± 7	9.1 ± 3	11.4 ± 8	35.5 ± 3
	48.0	···	···	···	51.3 ± 2	33.0 ± 5	4.6 ± 3	1.8 ± 3	21.6 ± 8
	64.0	···	···	···	1.9 ± 4	6.7 ± 5	0	0	8.0 ± 7

[a] Plants were sprayed to runoff with fungicide suspensions and inoculated about 2 hr later with *P. cubensis* (5 ± 1 sporangia per droplet per cotyledon). About 120 plants per treatment. Percentages were calculated from counts (at 7 days after inoculation) of number of cotyledons infected of those inoculated.

TABLE 3. Infectivity of three metalaxyl-sensitive (MS) and five metalaxyl-resistant (MR) isolates of *Pseudoperonospora cubensis* to cucumber plants at the two-true-leaf growth stage treated[a] with either metalaxyl or mancozeb at various concentrations

Fungicide	Fungicide conc. (μg a.i./ml)	Infected leaf area (% ± SD)							
		MS1	MS2	MS3	MR1	MR2	MR3	MR4	MR5
Metalaxyl	0	100	100	100	100	100	100	100	100
	3.12	14.8 ± 5	7.2 ± 3	56.8 ± 9	···	···	···	···	···
	6.25	0	0	44.9 ± 5	···	···	···	···	···
	12.50	0	0	39.3 ± 3	···	···	···	···	···
	25.00	0	0	30.0 ± 7	···	···	···	···	···
	31.25	0	0	25.0 ± 5	97.6 ± 3	100	87.0 ± 4	96.4 ± 6	100
	62.50	0	0	0	96.8 ± 2	100	74.4 ± 8	92.3 ± 5	98.5 ± 4
	125.0	···	···	···	89.2 ± 5	96.7 ± 3	70.0 ± 9	90.5 ± 6	92.5 ± 5
	250.0	···	···	···	81.5 ± 7	92.0 ± 4	42.9 ± 11	80.1 ± 8	91.8 ± 9
	500.0	···	···	···	70.9 ± 9	84.0 ± 6	28.2 ± 8	79.8 ± 10	85.6 ± 5
	750.0	···	· ·	···	14.4 ± 5	12.7 ± 8	5.1 ± 6	6.9 ± 9	8.9 ± 6
Mancozeb	2.0	64.3 ± 8	7.1 ± 3	89.5 ± 6	100	100	96.1 ± 4	98.0 ± 2	100
	4.0	50.0 ± 5	1.0 ± 1	69.9 ± 9	98.0 ± 2	100	90.2 ± 7	97.2 ± 7	98.1 ± 3
	8.0	9.4 ± 8	0	25.8 ± 12	···	···	···	···	···
	16.0	0	0	0	95.6 ± 4	97.9 ± 3	81.3 ± 6	86.4 ± 6	96.6 ± 3
	24.0	0	0	0	65.8 ± 6	87.0 ± 9	63.5 ± 8	52.5 ± 5	80.0 ± 8
	32.0	···	···	···	51.5 ± 7	52.0 ± 6	14.8 ± 11	19.3 ± 9	46.3 ± 7
	40.0	···	···	···	39.2 ± 10	38.7 ± 4	9.0 ± 4	9.0 ± 7	33.5 ± 9
	48.0	···	···	···	12.4 ± 6	30.0 ± 9	2.5 ± 7	2.6 ± 3	24.4 ± 9
	64.0	···	···	···	4.9 ± 4	8.6 ± 8	0	0	10.1 ± 6
	128.0	···	···	···	2.1 ± 2	5.6 ± 5	0	0	4.4 ± 5.5

[a] Plants (16 per treatment) were sprayed to runoff with fungicide suspensions and inoculated about 2 hr later with a suspension of 5,000 sporangia of *P. cubensis* per milliliter with a hand garden sprayer. Infected leaf area was measured at 7 days after inoculation.

TABLE 4. Approximate concentrations of metalaxyl and mancozeb required to reduce downy mildew by about 50% (ED_{50}) in cucumbers inoculated with either one of three metalaxyl-sensitive (MS) or five metalaxyl-resistant (MR) isolates of *Pseudoperonospora cubensis*

Plants at growth stage	Fungicide	Approximate ED_{50} value (μg a.i./ml)							
		MS1	MS2	MS3	MR1	MR2	MR3	MR4	MR5
Cotyledons	Metalaxyl	<3.12	<3.12	3.12–6.25	500–750	500–750	125–250	500–750	500–750
	Mancozeb	4–8	<2.0	4–8	24–32	32–40	24–32	24–32	24–32
Two-true-leaves	Metalaxyl	<3.12	<3.12	3.12–6.25	500–750	500–750	125–250	500–750	500–750
	Mancozeb	4.0	<2.0	4–8	32–40	32–40	24–32	24–32	24–32

[a] Data compiled from Tables 2 and 3.

and weighing. Experiments were repeated twice with similar results obtained. One set of experimental data is given.

RESULTS

MS isolates of *P. cubensis* were completely controlled by a foliar spray of metalaxyl at 6.25–62.5 μg a.i./ml in plants inoculated at either the cotyledonary or true-leaf stage. The concentration of metalaxyl required to reduce the symptoms by 50% (considered as ED_{50}) was <3.12, <3.12, and 3.12–6.25 μg a.i./ml for isolates MS1, MS2, and MS3, respectively, in either cotyledonary or true leaves (Tables 2–4). In contrast, the MR isolates produced disease in plants treated with metalaxyl concentrations as high as 750 μg a.i./ml in either cotyledonary (Table 2) or true leaves (Table 3). The ED_{50} values were 500–750 μg a.i. of metalaxyl per milliliter for all MR isolates except MR3, which produced an ED_{50} value of 125–250 μg a.i./ml (Table 4).

Toxicity of mancozeb to MR isolates on leaf surfaces of cucumbers was 3 to 16 times lower compared to its toxicity to MS isolates, depending on the pair of isolates (MS and MR) compared. Whereas the MS isolates were completely controlled by a foliar spray of mancozeb at 8–16 μg a.i./ml in plants at either the cotyledonary or true-leaf stage, the MR isolates still produced disease in plants sprayed with mancozeb at 48–128 μg a.i./ml. ED_{50} values for the control of MS isolates by mancozeb ranged between <2 and 4–8 μg a.i./ml in either cotyledonary or true leaves, as against a range of 24–40 μg a.i./ml for MR isolates (Table 4). Amongst strains tested, MS2 was the most sensitive to both metalaxyl and mancozeb, whereas MR2 was most resistant to both metalaxyl and mancozeb (Tables 2–4). Whereas MS2 was completely eliminated from leaves treated with either metalaxyl at 6.25 μg a.i./ml or with mancozeb at 8 μg a.i./ml, the MR2 was still active in leaves treated with either metalaxyl at 750 μg a.i./ml or with mancozeb at 128 μg a.i./ml. In all the above-mentioned experiments (Tables 2 and 3), symptom production in leaves or cotyledons was always followed by fungal sporulation subsequent to a moist period of 24 hr in darkness at 20 C.

DISCUSSION

According to Fry (2, page 280) resistance to ethylenebisdithiocarbamate fungicides has been rare in the field. Some fungi can be "trained" or "adapted" to these compounds in laboratory experiments, but such isolates have limited pathogenicity in the field. The only documented case was reported by Lorbeer and Ellerbrock (5) who observed a decline in the effectiveness of mancozeb in controlling *Botrytis squamosa* in onions in New York commencing in 1972. They showed that fungal isolates collected from onions sprayed with mancozeb in 1975 exhibited tolerance to mancozeb on onion leaf surfaces compared to an isolate collected in 1964.

Results presented in this paper show that while some field isolates of *P. cubensis* were highly sensitive to mancozeb in laboratory experiments, others could produce disease and propagate in plants treated with mancozeb. Resistance to mancozeb was associated with resistance to metalaxyl. All MR isolates were resistant to mancozeb, but the three MS isolates were not, which suggests existence of cross-resistance between metalaxyl and mancozeb. Our previous data showed (1) that MR isolates of *P. cubensis* were also resistant to four other systemic fungicides (propamocarb, vinicur, Aliette, and SAN 371F). This broad spectrum of cross-resistance suggests that reduced fungal membrane permeability may be responsible for resistance of MR strains to all chemicals. The obligatory nature of *P. cubensis* limits the biochemical experimentation required to confirm this.

Our results have practical and theoretical implications. In cucumber production areas where MR strains of *P. cubensis* predominate it might be advisable to use strengthened mixtures of metalaxyl-mancozeb (7) or triphenyltin acetate, chlorothalonil, and folpet which retained efficacy against MR isolates in the laboratory and in the field (Y. Samoucha, *unpublished*).

Theoretical models developed to predict the buildup of fungal subpopulations resistant to site-specific (systemic) fungicides under various control strategies (3,4,8) should consider the possible existence of cross-resistance to protectant fungicides.

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