Synergy Between Metalaxyl and Mancozeb in Controlling Downy Mildew in Cucumbers

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ABSTRACT

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A single foliar spray of either metalaxyl, mancozeb, or a mixture of both was applied to cucumber plants in growth chambers or in the field 1 day before inoculating with metalaxyl-sensitive or metalaxyl-resistant strains of *Pseudoperonospora cubensis*. Disease records taken at 7 days after inoculation showed a greater efficacy of mixtures over that of each fungicide separately at corresponding concentrations. Synergy factor values (the relative increase in control efficacy) obtained for the metalaxyl-sensitive and -resistant strains in growth chambers were 1.09–1.36 and 1.12–2.90, and in the field 1.06–1.36 and 2.66–24.41, respectively.

Additional key words: chemical control strategies, epidemiology, systemic fungicides.

Data on the efficacy of fungicide mixtures in controlling fungal plant pathogens are virtually lacking in the literature. Prepacked mixtures of a systemic (site-specific) fungicide and a protectant fungicide are commercially available and used by growers to delay the buildup of fungal strains resistant to systemic fungicides (3–5,8). In spite of the practical and theoretical importance of using such mixtures, very little data are available on the control efficacy of such mixtures. Recently, Gisi et al (3) showed that mixtures of SAN 371 F-mancozeb, were more effective in controlling *Phytophthora infestans* on potatoes and *Plasmopara viticola* on grapes than each fungicide used alone.

The present paper supplies data on the separate and combined efficacy of metalaxyl and mancozeb in controlling downy mildew in cucumbers incited by either metalaxyl-sensitive or metalaxyl-resistant strains of *Pseudoperonospora cubensis.*

MATERIALS AND METHODS

Growth chamber experiments. *Cucumis sativus* L. 'Elem,' which is highly susceptible to downy mildew, was used. Plants were grown, 10 plants per pot, in 10-cm-diameter plastic pots filled with a mixture of sandy loam, peat, and vermiculite (2:1:1, v/v) in the greenhouse (20–34 C), and were used about 10 days after sowing when cotyledonary leaves were about 3 cm long. Plants (four pots per treatment) were sprayed to run-off with fungicidal suspensions on adaxial leaf surfaces with the aid of a hand garden sprayer (about 10 ml per 40 plants), and used for inoculation ~24 hr later. Inoculation was done by placing one 10-μl droplet of sporangial suspension (prepared in ice-cooled double distilled water) containing 5 ± 1 sporangia on the adaxial (upper) surface of each cotyledon. Droplets were produced with the aid of a Nichiryo dispensing syringe (model 8100, Nichiryo Co., Chiyoda-Ku, Tokyo). Plants were incubated in a dew chamber (Percival, model D-60) at 17 C in the dark for about 20 hr and then in a 25 C growth cabinet (50–60% RH, 14 hr of light per day at ~120 μEinsteins$\cdot m^{-2} \cdot s^{-1}$ from cool-white fluorescent lamps) for 7 days when the number of cotyledons infected was recorded.

The metalaxyl-sensitive strain MS_3 of *P. cubensis* used in this study was isolated from cucumbers growing in the field at Kadima in summer 1983. The metalaxyl-resistant strain MR_1 was isolated from cucumbers in a plastic house in Hadera in winter 1979 (7). Strains were propagated on cucumber plants in separate growth chambers to avoid cross contamination.

Field experiments. Field experiments were conducted during August–September 1983 in two 250 m^2 plots in Kefar-Abraham (5 km east of the campus). Cucumber plants (cultivar Elem) with two leaves in peat pots were transplanted in the field (both plots) on 10 August. On 29 August, when the plants in plot 1 had developed about 12 leaves, they were sprayed with fungicidal suspensions with the aid of a backpack mist blower (model Nuvola L-80, Cifarilli Rafalle, Italy, 0.5 L per 10 plants per treatment). On 30 August at 2100 hours plants were sprayed on adaxial leaf surfaces, with the aid of a hand garden sprayer, with sporangial suspensions (500 sporangia per milliliter in ice-cooled double distilled water) of the MS_3 strain of *P. cubensis*. Plants in plot 2 (20 per treatment) were similarly sprayed with fungicidal suspensions on 11 September when they had developed about 24 leaves. Plants in plot 2 were similarly inoculated on the same day at 2100 hours with the MR_1 strain of the fungus as described for plot 1.

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Disease was recorded in both plots 7 days after inoculation. Leaves were detached and brought to the laboratory where the infected area of each leaf was determined by tracing it on a transparent paper, cutting out the delineated area, and weighing it.

Synergy factor determination. Synergy factor in both growth chamber and field experiments was calculated by using the formula:

$$\text{synergy factor (Sf)} = M/[P + 0.01S(100-P)]$$

in which M, P, and S represent percentage disease control incited by mixtures, mancozeb, and metalaxyl, respectively. An Sf value of 1 indicates additive action between fungicides in mixture, while an Sf value of >1 indicates synergistic action. The formula is based on the findings (Y. Cohen, *unpublished*) that the protectant fungicide (mancozeb) inhibits zoospore release and cyst germination of *P. cubensis*, whereas the systemic fungicide (metalaxyl) inhibits postinfectional stages of the pathogen's development.

RESULTS

In growth chamber experiments, disease induced by the metalaxyl-sensitive strain MS_3 of *P. cubensis* was controlled by 56–58 and 100% in plants sprayed with 0.625 and 6.25 μg a.i. metalaxyl per milliliter, respectively. Mancozeb at 4 and 16 μg a.i./ml reduced disease by 40–45 and 100%, respectively (Table 1). The efficacy of fungicides in mixtures was greater than their individual efficacies combined. With 2 μg per milliliter of mancozeb in the mixture, synergy factors ranged 1.07–1.14, and with 4 μg of mancozeb per milliliter the synergy factor ranged 1.09–1.36, with higher values observed at lower metalaxyl concentrations (Table 1). Table 2 shows that about 45 and 100% control of the disease incited by the metalaxyl-resistant strain MR_1 was achieved by metalaxyl at 62.5 and 187.5–250 μg/ml, respectively. Interestingly enough, more mancozeb was required to control this strain than the MS_3 strain. Fifty and 100% control of the disease in cotyledons were achieved by mancozeb at 8 and 20–32 μg/ml, respectively. Disease incited by the MR_1 strain was far more efficiently controlled by mixtures than by the individual fungicides exhibiting synergy factors of 1.12–2.90, and 1.19–2.84 in experiments 1 and 2, respectively. Sf values were larger at 4 than at 8 μg a.i. of mancozeb per milliliter, but in both cases Sf values increased with decreasing concentration of metalaxyl in the mixtures.

TABLE 1. The effect of a single foliar spray with metalaxyl, mancozeb, or a mixture of both on the infectivity of a metalaxyl-sensitive strain MS_3 of *Pseudoperonospora cubensis* to cucumbers under laboratory conditions[a]

Fungicide(s) and conc. (μg a.i./ml)		Experiment 1		Experiment 2	
Metalaxyl	Mancozeb	Cotyledons infected (% ± SD)	Synergy factor	Cotyledons infected (% ± SD)	Synergy factor
0	0	100	-	100	-
0.625	0	42 ± 3	-	44 ± 3	-
1.25	0	27 ± 6	-	28 ± 8	-
2.5	0	15 ± 5	-	17 ± 5	-
6.25	0	0.0	-	0.0	-
0	2.0	64 ± 2	-	73 ± 3	-
0	4.0	55 ± 7	-	60 ± 3	-
0	8.0	11 ± 3	-	13 ± 4	-
0	16.0	0.0	-	0.0	-
0.625	2.0	22 ± 2	1.07	24 ± 7	1.12
1.25	2.0	9 ± 4	1.10	9 ± 6	1.14
2.5	2.0	1 ± 1	1.31	1 ± 1	1.13
0.625	4.0	0.0	1.18	0.0	1.36
1.25	4.0	0.0	1.09	0.0	1.20
2.5	4.0	0.0		0.0	1.11

[a]The experiment was conducted with about 40 plants (80 cotyledons) per treatment. The synergy factor was calculated by using the formula described in Materials and Methods.

TABLE 2. The effect of a single foliar spray with metalaxyl, mancozeb, or a mixture of both on the infectivity of a metalaxyl-resistant strain MR_1 of *Pseudoperonospora cubensis* to cucumbers under laboratory conditions

Fungicide(s) and conc. (μg a.i./ml)		Experiment 1		Experiment 2	
Metalaxyl	Mancozeb	Cotyledons infected (% ± SD)	Synergy factor	Cotyledons infected (% ± SD)	Synergy factor
0	0	100	-	100	-
31.25	0	89 ± 4	-	87 ± 5	-
62.5	0	55 ± 2	-	56 ± 6	-
125.0	0	24 ± 5	-	32 ± 8	-
187.5	0	0.0	-	16 ± 9	-
250.0	0	0.0	-	0.0	-
0	4.0	79 ± 9	-	81 ± 7	-
0	8.0	46 ± 6	-	50 ± 3	-
0	16.0	18 ± 4	-	21 ± 7	-
0	20.0	0.0	-	5 ± 5	-
0	32.0	0.0	-	0.0	-
31.25	4.0	14 ± 7	2.90	16 ± 6	2.84
62.5	4.0	4 ± 8	1.70	4 ± 8	1.76
125.0	4.0	1 ± 0	1.22	0.0	1.35
31.25	8.0	8 ± 3	1.56	6 ± 9	1.66
62.5	8.0	1 ± 1	1.33	0.0	1.39
125.0	8.0	0.0	1.12	0.0	1.19

TABLE 3. The efficacy of a single foliar spray with metalaxyl, mancozeb, or a mixture of both in controlling downy mildew in cucumbers incited by the metalaxyl-sensitive strain MS_3 of *Pseudoperonospora cubensis* in the field conditions[a]

Fungicide(s) and conc. (μg a.i./ml)		Leaf area infected	Synergy
Metalaxyl	Mancozeb	(% ± SD)	factor
0	0	95 ± 3	-
1	0	90 ± 5	-
2	0	80 ± 7	-
4	0	66 ± 3	-
8	0	48 ± 5	-
16	0	9 ± 5	-
32	0	0.0	-
0	4	71 ± 7	-
0	8	50 ± 4	-
0	12	34 ± 5	-
0	16	25 ± 5	-
0	24	10 ± 9	-
0	32	7 ± 3	-
0	48	0.0	-
1	4	58 ± 3	1.36
1	8	41 ± 7	1.15
1	12	20 ± 6	1.20
2	8	25 ± 8	1.33
2	16	12 ± 6	1.11
2	24	4 ± 2	1.06
4	16	5 ± 6	1.16
4	32	1 ± 1	1.04
8	4	12 ± 5	1.41
8	8	1 ± 0.3	1.30
16	4	5 ± 2	1.32
16	8	0.0	1.06

[a] The experiment was conducted with ten 12-leaf plants per treatment. Plants were sprayed with fungicide suspensions at 0900 hours (29 August 1983) and inoculated at 2100 hours (30 August 1983). Disease records were taken of 120 leaves per treatment 1 wk later.

TABLE 4. The efficacy of a single foliar spray with metalaxyl, mancozeb, or a mixture of both in controlling downy mildew in cucumbers incited by the metalaxyl-resistant strain MR_1 of *Pseudoperonospora cubensis* under field conditions

Fungicide(s) and conc. (μg a.i./ml)		Leaf area infected	Synergy
Metalaxyl	Mancozeb	(% ± SD)	factor
0	0	100	-
31.25	0	96 ± 8	-
62.5	0	85 ± 5	-
125.0	0	79 ± 6	-
250.0	0	55 ± 3	-
500.0	0	27 ± 6	-
750.0	0	4 ± 2	-
0	4	98 ± 2	-
0	8	90 ± 5	-
0	16	79 ± 7	-
0	24	54 ± 5	-
0	32	32 ± 4	-
0	48	15 ± 6	-
15.62	2	27 ± 5[a]	24.41
31.25	4	15 ± 4	14.36
62.5	8	4 ± 2	4.08
125.0	16	0.0	2.66

[a] Based on the assumption that either 15.62 μg a.i. metalaxyl per milliliter or 2 μg a.i. mancozeb per milliliter would result in 99% infected leaf area. The experiment was conducted with twenty 24-leaf plants per treatment as described in Table 3.

Performance of either fungicide in the field was lower than its performance in growth chambers. About 50% reduction in disease development incited by the metalaxyl-sensitive strain was observed in plants treated with 8 μg a.i./ml of either metalaxyl or mancozeb, while complete control was achieved by 32 and 48 μg a.i./ml, respectively (Table 3). When mixed, the fungicides controlled the disease incited by the MS_3 strain more effectively. Thus, a mixture of 8 μg a.i./ml metalaxyl and 8 μg a.i./ml mancozeb gave 99% control as against about 50% control achieved with either fungicide at 8 μg a.i./ml when used alone (Sf = 1.30) (Table 3). Control was complete with a mixture of 16 μg a.i./ml metalaxyl and 8 μg a.i./ml mancozeb (Sf = 1.06). At a fixed concentration of metalaxyl, the synergy factor increased with decreasing mancozeb concentration, and vice versa.

A strong synergistic effect (Sf values of 2.66–24.41) of the two fungicides was observed in the field when mixtures were applied to control the disease incited by the metalaxyl-resistant strain MR_1. Thus, whereas metalaxyl alone at 125 μg a.i./ml and mancozeb alone at 16 μg a.i./ml gave about 20% control of the disease, their mixture (same concentrations) resulted in a complete control of the disease (Sf = 2.66). Similarly, metalaxyl and mancozeb at 62.5 and 8 μg a.i./ml gave about 15 and 10% control, respectively, when used singly and 96% control when used as a mixture (Sf = 4.06) (Table 4). Highest synergistic action in this experiment was observed with the least concentrated fungicidal mixture.

DISCUSSION

Results presented in this paper show that a foliar spray of metalaxyl–mancozeb mixtures provided a much better control of downy mildew in cucumbers than either metalaxyl or mancozeb alone. This was true under both growth chamber and field conditions when either a metalaxyl-sensitive or a metalaxyl-resistant strain was used to induce the disease. The relative increase in control efficacy of mixtures was found to be 1.06–24.41 times greater than that of the individual fungicides combined. These findings stand in accordance with those of Gisi et al (3). They showed a synergy factor of 1.4–2.7 and 3.6–14.6 for SAN 371 F-mancozeb mixtures tested on potatoes inoculated with metalaxyl-sensitive and metalaxyl-resistant isolates of *P. infestans*, respectively. However, Gisi et al (3) found no synergy between metalaxyl and mancozeb in control of grape downy mildew in the field.

The mechanism governing synergy between fungicides is not known. A reasonable speculation would be (K. J. Leonard, *personal communication*) that since (in this case) metalaxyl and mancozeb have a different mode of action (the former mostly inhibits fungal RNA synthesis [2] while the latter impairs energy production [6]), exposure of sporangia to sublethal concentrations of one fungicide weakens them to an extent that sublethal doses of the second fungicide will be detrimental. Further study is currently being undertaken to elucidate this mechanism.

Mathematical models developed to predict the buildup of fungal subpopulations resistant to systemic site-specific fungicides under various control strategies, including the use of mixtures with a protectant (4,5,8), suffered from a lack of information as to the combined controlling effect of fungicidal mixtures (relative to their spatial effect) on the sensitive and resistant subpopulations. Results presented in this paper would assist the refinement of such models. Our results also show that a fungal subpopulation resistant to metalaxyl could still be controlled by metalaxyl if appropriate mixtures with mancozeb are applied.

The fact that our MR_1 strain was more tolerant to mancozeb than the MS_3 strain needs more study for clarification. It indicates, however, that the resistance of MR_1 and MR_2 to prothiocarb, SAN 371 F, vinicur, and Aliette (1) may result from a common mechanism.

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