Control of Sunflower Downy Mildew with Metalaxyl

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ABSTRACT

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Dressing of sunflower seeds with metalaxyl at 1, 2, or 4 g a.i./kg prevented *Plasmopara helianthi* sporulation on seedlings inoculated by dipping the roots into a zoosporangial suspension. Washing seedlings with water before inoculation did not influence results, suggesting a systemic activity of the fungicide in the seedlings. One or two drenches of soil in pots with metalaxyl at concentrations of 125, 250, or 500 mg a.i./L reduced symptom severity in *P. helianthi*-infected seedlings and allowed plants to resume normal growth. Symptom remission in infected plants was also achieved by fungicide sprays in the field. Highest incidence of symptom remission occurred with three sprays at a concentration of 520 mg a.i./L and two or three sprays at a concentration of 1,040 mg a.i./L.

Downy mildew (DM) caused by *Plasmopara helianthi* Novot. severely affects sunflower (*Helianthus annuus* L.) in Spain (8,13) as well as in other countries (13). Primary infections by the pathogen result in systemic colonization of plants and are far more frequent than secondary local infections, which may not reduce seed yields but can be important in the spread of the pathogen through the seeds (4).

The disease is controlled mainly by resistant cultivars, the use of which may be restricted by new, more virulent races of *P. helianthi* (20,21). However, the development of systemic fungicides effective against Oomycetes opens the possibility of chemical control. Recently, work done with Ridomil 25 WP (5,14,15) and Apron 35 SD (1,7,18) has shown the efficacy of seed treatments with metalaxyl [*N*-(2,6-dimethyl-phenyl)-*N*-(2'-methoxyacetyl)-alanine methyl ester] in the control of sunflower DM. Foliar sprays with metalaxyl controlled several locally infecting DM (2,10,12,19), but were only partially effective against a systemic DM affecting maize (9).

The objective of the research reported in this paper was to determine the efficacy of soil drenches and foliar sprays with metalaxyl in the therapy of sunflower DM. An effort was also made to determine whether the effectiveness of seed treatments with metalaxyl is the result of a protective or a systemic action. A preliminary report of this work has been published (5).

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MATERIALS AND METHODS

Peredovik, a sunflower cultivar susceptible to DM, and metalaxyl (Ridomil 25 WP) were used in two experiments (I,II) on seed treatment, one on soil drench and one on foliar spray.

Seed treatment and soil drench experiments were carried out in growth chambers adjusted to 20 ± 2 C by day and 18 ± 2 C by night, and to a 12-hr photoperiod of 15 klux. Seedlings were inoculated with a suspension of 5×10^4 zoosporangia per milliliter of distilled water for 6–7 hr at 20 ± 2 C. The seedlings were then planted in 10-cm-diameter plastic pots containing perlite or an artificial, nonsterile soil mixture (peat moss:sand:silt, 1:2:2 by volume).

Seed treatment. One-hundred gram lots of seeds were treated with metalaxyl at the rates of 0, 1, 2, and 4 g a.i./kg in 70 ml of distilled water. The fungicide was applied by agitating the seeds in a glass jar until most of the fungicide covered the seeds (6).

In experiment I, 3-day-old seedlings with 1- to 2-cm-long rootlets were inoculated by dipping only the rootlet into the inoculum suspension. Just before inoculation, about half of the seedlings were kept for 2 hr in a stream of tap water. For each treatment combination, five to seven seedlings were planted in each of 10 pots. Disease reaction was assessed by sporulation on cotyledons 11 days after inoculation. Sporulation was induced by placing the seedlings in a saturated atmosphere 24 hr before assessment.

In experiment II, seedlings from metalaxyl-treated (concentration as stated above) and untreated seeds were grown for 7 days. They were then uprooted, washed in a stream of tap water for 2 hr, inoculated, and repotted at three to four seedlings per pot. For each fungicide concentration and the untreated seeds, seedlings were inoculated in separate beakers; there were 11 pots per treatment. Additional seedlings from untreated seeds, which were inoculated simultaneously in the beakers with the treated seedlings, served as checks for the direct activity of the fungicide on the inoculum. Those seedlings were planted in six pots per treatment with three to four seedlings per pot. Disease reaction was assessed by the occurrence of symptoms of systemic infection 1 mo after inoculation.

Soil drench. Three-day-old seedlings were inoculated by the whole seedling inoculation method (3) and planted three to four per pot. Soil drenches at the rate of 40 ml/pot (500 ml of soil mixture) were done with an aqueous solution of metalaxyl at concentrations of 0, 125, 250, or 500 mg a.i./L. Drenches were done 11 days or 11 and 18 days after inoculation. Eight pots were used for each treatment combination. When the first drench was applied, seedlings were at the two-leaf stage and systemic symptoms had not developed. Assessment of disease reaction was made 4 wk after the first drench.

Foliar spray. A field of sunflowers with more than 90% natural systemic infections was thinned to leave 100% incidence of the disease. Experimental plots consisted of three rows 5 m long and 75 cm apart, with about 25-cm plant spacing.

Plants were sprayed with an aqueous solution of metalaxyl at concentrations of 0, 260, 520, or 1,040 mg a.i./L. Sprays were applied one to three times for every concentration at 12-day intervals with a portable, single-nozzle sprayer delivering 500 L/ha. The first spray was done 21 days after emergence when plants were at the six-leaf stage. Treatments were arranged in a randomized complete block design with six replications.

The percentage of plants with symptom remission in leaves was determined 2 mo after the first spray when plants had completed the blooming stage. One week later, at initiation of maturity, the head diameter and percentage of horizontal heads (a symptom of sunflower DM) were determined in a random sample of 17 plants per plot. Finally, at harvest, the head diameter in 15 random plants and the average seed yield per plant were determined in each plot. Analyses of variance were performed with all data, the percentage values being arc-sin transformed before analysis. Mean comparisons were done following the Student-Newman-Keuls' test (17) at $P = 0.01$.

RESULTS

Seed treatment. In experiment I, infection in the untreated control was 88.3%. No sporulation developed on any of the metalaxyl-treated seedlings, either washed or unwashed (Table 1). In experiment II, however, only the rates of 2 and 4 g a.i./kg reduced the percentage of infected seedlings to a significant extent, as compared with the untreated, separately inoculated control (Table 1).

Soil drench. Fungicidal drenches of the soil in pots reduced symptom severity in inoculated seedlings. Ninety percent of the control seedlings were stunted and chlorotic, showing in the leaves the mosaic pattern typical of the disease (Table 2). By contrast, seedlings in pots drenched with each concentration of metalaxyl were not stunted, but showed only a light chlorosis around the veins in the second pair of leaves, while the first pair was mostly necrotic. Furthermore, the incidence of such chlorosis decreased with two drenches of 250 mg a.i./L and was the lowest with two drenches of 500 mg a.i./L (Table 2).

Soil drenches with metalaxyl induced necrosis of leaf margins and brown necrotic spots 2–3 cm in diameter in the interveinal spaces of leaves. The severity of symptoms of phytotoxicity increased with the fungicide concentration, with the number of drenches, and in a basipetal direction in the seedlings (the third pair of leaves was only occasionally affected). Phytotoxic symptoms did not, however, influence the normal growth of the seedlings.

Foliar spray. No phytotoxic effects were observed in this experiment. Plants sprayed with metalaxyl showed a remission of DM symptoms, which consisted of a chlorosis restricted to the vicinity of the leaf veins (Fig. 1). This remission was more evident in the upper leaves of plants sprayed two to three times at 12-day intervals with fungicide concentrations of 1,040 or 520 mg a.i./L. Remission of symptoms was transient in nature because, by the time of the third spray, the typical chlorotic mosaic occurred in leaves above those that had showed symptom remission. Results on symptom remission and average seed yield per plant are summarized in Figure 2.

Greatest incidence of symptom remission occurred with two or three sprays at 1,040 mg a.i./L and three sprays at 520 mg a.i./L. One spray at 260 mg a.i./L or 520 mg a.i./L did not reduce symptom severity as compared with the unsprayed control. Results on average seed yield per plant followed the same

Table 1. Effect of seed treatment with metalaxyl on the incidence of downy mildew in sunflower seedlings

	Experiment I[x]				Experiment II[y]			
	Washed seedlings		Unwashed seedlings		Treated seedlings		Untreated seedlings	
Rate (g a.i./kg)	Inoculated (no.)	Sporulating (%)	Inoculated (no.)	Sporulating (%)	Inoculated (no.)	Infected (%)	Inoculated (no.)	Infected (%)
0	60	88	...	...	42	86 a[z]	42	86 a
1	62	0	56	0	33	52 b	19	58 b
2	54	0	64	0	42	21 c	18	61 b
4	59	0	65	0	45	11 c	17	70 ab

[x]Washed, 3-day-old seedlings were kept in a stream of tap water for 2 hr before inoculation. Sporulation on cotyledons was induced by placing in a saturated atmosphere 10 days after inoculation.
[y]Seven-day-old seedlings were washed in a stream of tap water for 2 hr before inoculation. Fungicide-treated and untreated seedlings were inoculated together in the same container. Symptoms of DM were assessed 1 mo after inoculation.
[z]Values followed by same letter within rows or columns are not significantly different ($P = 0.05$), according to the χ^2 interaction test.

Table 2. Effect of soil drenches with metalaxyl in the therapy of sunflower downy mildew[x]

		Seedlings		
Concentration (mg a.i./L)	No. of drenches	Inoculated (no.)	With symptoms in all leaves (%)[y]	With symptoms only in the second pair of leaves (%)[y]
0	...	30	90	...
125	1	29	0	41 a[z]
	2	28	0	43 a
250	1	32	0	41 a
	2	30	0	17 b
500	1	28	0	25 ab
	2	26	0	6 b

[x]Untreated, 3-day-old seedlings were inoculated by the whole seedling inoculation method (3) and grown in pots. Soil drenches were applied to the pots 11 days or 11 and 18 days after inoculation.
[y]The effect of the soil drenches was assessed 4 wk after the first application. Seedlings in pots drenched with metalaxyl had the first pair of leaves mostly necrotic and showed light chlorosis around the veins in leaves of the second pair.
[z]Values followed by same letter are not significantly different ($P = 0.05$), according to the χ^2 interaction test.

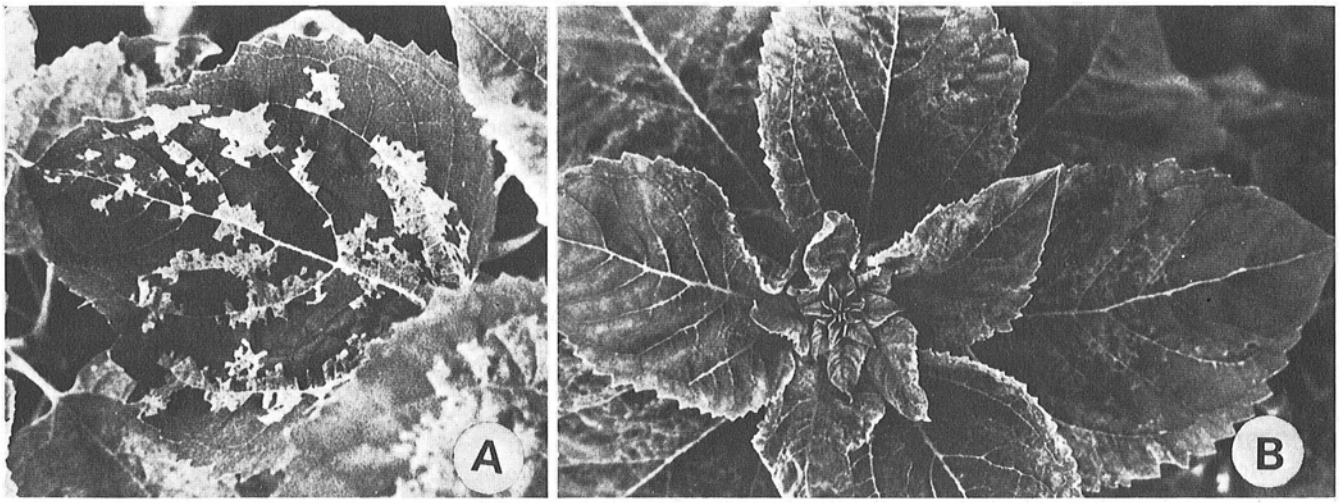

Fig. 1. Symptom remission in *Plasmopara helianthi*-infected sunflowers: **(A)** Unsprayed control plant showing the characteristic chlorotic pattern. **(B)** Restricted chlorosis in plant sprayed with metalaxyl.

Table 3. Effect of foliar sprays with metalaxyl on the incidence of horizontal heads and on head diameter in *Plasmopara helianthi*-infected sunflower

Concentration (mg a.i./L)	No. of sprays[x]	Horizontal heads (%)[y]	Head diameter (cm) at	
			Initiation of maturity	Harvest
0	...	62 ab[z]	5.7 a	...
260	1	60 ab	5.9 a	5.3 a
	2	67 a	8.7 c	7.4 b
	3	76 a	10.6 d	8.9 cd
520	1	56 ab	7.0 b	5.6 a
	2	44 abc	10.4 d	9.6 de
	3	42 bc	12.0 e	10.9 ef
1,040	1	57 ab	9.0 c	7.6 bc
	2	22 c	12.3 e	11.0 f
	3	7 d	13.4 f	11.3 f

[x]Sprays were applied at 12-day intervals beginning 21 days after seedling emergence, with plants at the six-leaf stage.
[y]The horizontal head is typical of systemic infections by *P. helianthi*.
[z]Values followed by same letter within columns are not significantly different ($P = 0.01$), according to the Student-Newman-Keuls' test.

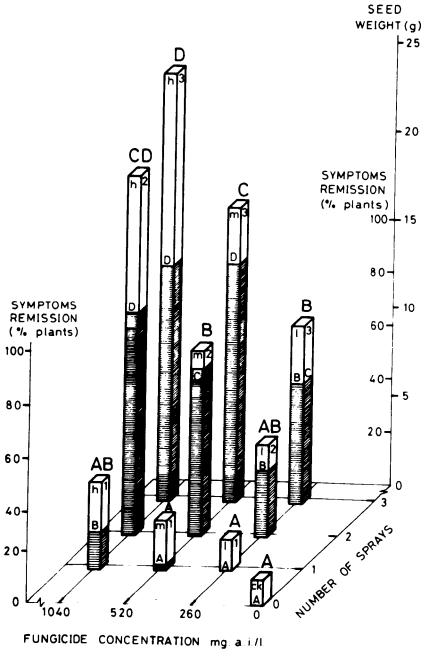

Fig. 2. Symptom remission (filled bars) and seed weight (whole bars) in *Plasmopara helianthi*-infected sunflowers sprayed with metalaxyl (l = 260, m = 520, h = 1,040 mg a.i./L). Capital letters over bars indicate statistical significance.

pattern as those on symptom remission (Fig. 2).

Average incidence of horizontal heads at initiation of maturity and head diameter at initiation of maturity and harvest are presented in Table 3. Two or three sprays at 1,040 mg a.i./L and three sprays at 520 mg a.i./L were again the most effective treatments. Also, incidence of horizontal heads at initiation of maturity and mean head diameter at initiation of maturity and harvest followed a similar trend.

DISCUSSION

The effectiveness of seed treatment with metalaxyl in the control of sunflower DM was first shown by Sackston (14,15), using the whole seedling inoculation method (3). This might be interpreted as a protection from infection by the fungicide. Our results with washed seedlings in the seed treatment experiments (Table 1) suggest a systemic activity of the compound in the seedlings, possibly after its absorption by the rootlets during germination. Alternatively, a direct fungicidal activity on the inoculum suspension may occur, although that would contrast with results reported with other Oomycetes (16).

In experiment II, the inoculation of seedlings from treated and untreated seeds together in the same beakers appeared to reduce the incidence of infection in the untreated seedlings as compared with that in the untreated, separately inoculated controls (Table 1). This suggests the possibility that metalaxyl diffused into the inoculum suspension from treated seedlings and interfered with the infection process.

The incidence of infection in seedlings from treated seeds in experiment II also contrasts with the lack of infections found with the same rates of the fungicide in experiment I (Table 1). Those results indicate that as the time between seed treatment and seedling inoculation increases, the effectiveness of metalaxyl decreases. That loss in effectiveness may be important in the field; however, the short period of susceptibility to systemic symptoms (3) probably occurs when metalaxyl is still active.

Results of soil drenches and foliar sprays indicated the effectiveness of metalaxyl in the therapy of systemic infections by *P. helianthi*. This contrasts with results obtained with the same compound in the therapy of systemic infection of maize by sugarcane DM (9). The transient nature of symptom remission that we observed between the second and third sprays suggests a fungistatic activity of the compound.

The phytotoxic symptoms induced by metalaxyl in sunflower seedlings after soil drenches did not appear in plants sprayed in the field, though relatively high concentrations were used. The data suggest that a single spray of a metalaxyl concentration higher than 1,040 mg a.i./L will bring complete recovery of sunflower plants systemically infected by *P. helianthi* (Fig. 2, Table 3). Such a spray would probably be most effective if applied as soon as symptoms develop in the plants.

Seed dressing is the most effective and economically feasible way to control sunflower DM with metalaxyl (1,7,18). However, the therapy achieved with soil drenches and especially with foliar sprays might be useful in experimental fields and breeding nurseries. In any case, the risk of *P. helianthi* developing resistance to metalaxyl should be considered in the strategies of using it to control sunflower DM. Resistance to metalaxyl in other Oomycetes has already been reported (10,11).

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