Metalaxyl for Control of Downy Mildew of Pea Caused by *Peronospora viciae*

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ABSTRACT

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Laboratory and field studies were done to evaluate metalaxyl for control of downy mildew (*Peronospora viciae*) of pea. Colonized areas on leaflets were smaller on plants sprayed with metalaxyl 2 days before incubation than on plants sprayed 6 days before inoculation. Similarly, areas colonized were smaller on leaflets treated 1–3 days after inoculation than on those treated 5–7 days after inoculation. Volatiles from a metalaxyl solution in a closed container prevented sporulation at concentrations of 200 μg/ml. In the field, metalaxyl at 0.01 g (a.i.)/m^2 in 50 ml of water (100 g [a.i.]/ha in 500 L of water) sprayed 3 days after inoculation gave best control of pea downy mildew. However, metalaxyl incorporated in soil at 2.0 kg (a.i.)/ha gave best control when applied at the same time as inoculation or 3 days later.

Additional key words: Ridomil, volatile activity

Pea downy mildew caused by *Peronospora viciae* (Berk.) Casp. has been reported from almost all of the pea-growing regions in the world. The most recent epiphytotic was in Wisconsin in 1974 (4). Nonsystemic fungicides have had little success in disease control (1,7), and until recently few fungicides were effective against the Peronosporales. Before 1978 the main exception was streptomycin, which was successfully used against downy mildews such as those on hops and tobacco (2,3).

Novel compounds exhibiting systemic activity have recently been found to control these pathogens. The most promising is metalaxyl. Urech et al (11) reported that metalaxyl controlled *Pseudoperonospora* on hops, *Peronspora* on tobacco, and *Sclerospora* on cereals. It can be applied to foliage or soil and is transported acropetally within the apoplast (10).

Our studies concern the protective and curative effects of metalaxyl against *P. viciae* of peas (*Pisum sativum* L.). We applied the fungicide to foliage or soil and examined its effects on the colonization of tissue and on sporulation.

MATERIALS AND METHODS

Pathogen maintenance and inoculum preparation. *P. viciae* was maintained on the pea cultivar Skagit in a growth room at 18–20 C with 12 hr of light under white fluorescent tubes (about 10 w/m^2) alternating with 12 hr of darkness. Seeds were sown in steam-sterilized compost (John Innes No. 2) in plastic trays (350 × 220 × 50 mm). Seedlings were inoculated at the two to three leaf stage and immediately covered with a transparent plastic tray lid. Inoculated seedlings were kept in the dark for 20 hr at 12–15 C to ensure infection. After the tray lid ventilators were opened, the trays returned to the previous regime.

Seven days after inoculation the ventilators were again closed to create the high humidity necessary for sporulation. Sporangia were harvested at the time of maximum sporulation (10 days after inoculation) by excising infected seedlings just above soil level and immediately immersing them in distilled water.

The resulting sporangial suspension was filtered through 1-mm^2 mesh muslin and the sporangia were washed three times in water. The final suspension, in distilled water with 0.1% v/v Tween 20, was used to reinoculate Skagit plants or, after adjusting the concentration, to inoculate experimental seedlings. Plants were inoculated with suspensions containing 1×10^6 sporangia per milliliter in the growth room and with 9×10^4 sporangia per milliliter in the field.

Colonization and sporulation. The colonization of host tissues was assessed by clearing leaves and staining the fungal hyphae by a modification of Shobe and Lersten's technique (8). Leaflets were excised, fixed in absolute ethanol for 2 days, and transferred to a second aliquot of absolute ethanol for an additional 3 days. Leaflets then were placed in 0.6 M NaOH solution for 24 hr, washed in running water, replaced in absolute ethanol for 24 hr, and then placed in saturated chloral hydrate solution containing cotton blue stain (0.2 g/100 ml) at 45 C for 5 days.

The leaves were mounted on microscope slides in lactophenol and observed by Reichert phase contrast microscopy. A Whipple eyepiece grid (each square covered 4900 μm^2) was used to assess the area occupied by each colony. One or more hyphae in each grid square was counted as a positive record for that square. For each replicate, 10 leaflets were stained, and in each leaflet the first colony seen was measured.

The sporangia produced on infected tissues were counted by excising leaflets and immediately shaking them in water with a Rotamix shaker (Hook and

Table 1. Effect of metalaxyl on *Peronospora viciae* on pea plants maintained at 12–15 C with 12-hr photoperiods

Metalaxyl treatment	Mean leaf area colonized (mm^2)	Sporulation	
		Mean no. of sporangia/leaf	Sporangia/leaf area (no./mm^2)
Soil (0.05 [a.i.]/L)			
Wet, at planting	0	0	NA[a]
Dry, at planting	0	0	NA
Foliar (0.01/g [a.i.]/m^2)			
Days before inoculation			
6	0.78	20,000	51
2	0.54	11,000	32
Days after inoculation			
1	0.03	0	NA
3	0.17	0	NA
5	0.53	0	NA
7	...[b]	4,000	16
Unsprayed control	0.99	71,000	223

[a] Not applicable.
[b] Not examined.

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Tucker Ltd.). A haemocytometer was used to estimate the number of sporangiospores in the suspension. The area of each leaflet was determined by tracing the outline on paper, cutting it out, weighing it, and converting the weight to area.

The metalaxyl was a wettable powder containing 25% a.i. Suspensions were made up in distilled water in which the active ingredient had 0.71% solubility at 20 C.

Growth room experiments. *Foliar application of metalaxyl.* Seeds of the pea cultivar Superb were sown in steam-sterilized compost in plastic trays (220 × 160 × 50 mm) and placed in the 18–20 C growth room regime before and after inoculation. Protectant treatments involved spraying plants at the second and third leaf stages 6 and 2 days, respectively, before inoculation by atomizing each tray with 30 ml of sporangial suspension. Curative sprays were applied 1, 3, 5, or 7 days after inoculation. Metalaxyl was used at the rate of 0.01 g (a.i.) in 50 ml of water per square meter. Control plants received no fungicide. The growth and sporulation of the pathogen in leaflets on the third node were assessed 6 and 10 days after inoculation, respectively.

Soil application of metalaxyl. Superb plants were grown in two trays, each filled with 1 L of compost. Metalaxyl solution, 50 ml at 0.05 g (a.i.)/L, was thoroughly mixed into each tray before sowing. In a second treatment, the rate was 200 g (a.i.) of metalaxyl added to 3 kg of dry sand, which then was mixed within a square meter of compost. All plants were inoculated and assessed as in the foliar treatment except that they were grown with minimal watering to prevent leaching of the fungicide.

Effect of metalaxyl vapor. Six Superb seedlings were sown in compost in each of 28 200-ml plastic cups. When 2-wk-old, plants were inoculated by spraying 10 ml of sporangial suspension into each cup. The cups were placed inside a second cup lacking drainage holes and then placed in plastic trays (350 × 220 × 50 mm) containing 0, 1, 10, 25, 100, 150, or 200 μg/ml of metalaxyl (four cups per tray, one tray per treatment). Trays were covered with a transparent plastic lid and all openings sealed with tape. The plants then were subjected to infection and maintenance procedures, except that lid ventilators were closed and sealed after 6 days. Ten days after inoculation, the number of sporangia on third leaflets from each of four plants per cup was counted.

Field experiments. *Foliar application of metalaxyl.* About 150 g of Superb seeds were sown in each of 24 field plots, 1.2 × 0.3 m, in four equal rows. Plants were inoculated at the third leaf stage with a 1 L/m^2 of sporangial suspension and immediately covered with polyethylene sheets. The sides and ends of the polyethylene were initially buried in the soil to minimize moisture loss. The sides of the polyethylene were lifted slightly to allow ventilation 24 hr after inoculation.

Each treatment comprised a single spray of 0.01 g (a.i.) of metalaxyl in 50 ml of water per square meter applied to four randomly selected replicate plots. Inoculated plots sprayed with 50 ml of water served as controls.

Plants were sprinkler irrigated, when required, at intervals of 5–6 days before inoculation. After inoculation, irrigation was done without removing the polyethylene sheets. The seventh day after inoculation, the sides of the sheets were reburied in soil to raise the internal humidity. Symptoms in field-grown plants were similar to those described by Hubbeling (5), except that in our experiments, sporulation on tendrils was common. On the 10th day, disease occurrence and severity were assessed by a slightly modified Hubbeling 0–4 scale in which 0 = no sporulation; 1 = local necrosis in leaves, no sporulation; 2 = limited production of sporangiophores, mostly confined to tendrils; 3 = abundant production of sporangiophores, mostly confined to tendrils; 4 = abundant production of sporangiophores on tendrils, leaves, and stems.

Soil application of metalaxyl. Superb seeds were sown in soil never previously treated with metalaxyl. Seed rate, plot size, inoculation, growing procedures, and disease assessments were as described. Each treatment consisted of one application of 0.2 g (a.i.) of metalaxyl per square meter. The fungicide for each plot was mixed with 50 g of dry sand, and the plants were gently shaken to dislodge fungicide from their surfaces. Each plot was watered with 2 L of water to ensure availability of the fungicide to the plant roofs.

RESULTS

Growth room experiments. Control of *P. viciae* was better in the growth room by preinoculation soil applications than by foliar sprays (Table 1). Both types of soil treatment prevented colonization. A foliar spray applied 6 days before inoculation was less effective than one applied just before inoculation. Colonization of leaves sprayed 6 days before inoculation was not significantly different from that of unsprayed plants. However, sporulation on leaves sprayed 6 days before inoculation was <25% that of the unsprayed control (Table 1).

The effectiveness of postinoculation foliar sprays depended on the length of time between inoculation and spray application. Sprays applied up to 3 days after inoculation severely limited the extent of pathogen spread within host tissues and also prevented sporulation. Colonization of leaves sprayed 5 days after inoculation was similar to that of leaves sprayed 2 days before inoculation, although no sporulation occurred on leaves sprayed 5 days after inoculation. The level of sporulation was very low even on leaves sprayed 7 days after inoculation.

Hyphae of *P. viciae* produced shorter and broader haustoria in tissue treated with metalaxyl than in unsprayed tissues. Several of these haustoria were branched at their distal ends compared with the normal unbranched, peglike haustoria.

Volatiles from a solution of metalaxyl reduced sporulation of the pathogen (Fig. 1). At 1 μg/ml sporulation was not reduced, but sporulation was clearly reduced when plants were maintained in a solution containing 10 μg/ml. When a concentration of 25 μg/ml was used, sporulation of *P. viciae* was about 50% that of the control treatment, and at greater concentrations reduction in sporulation was proportional.

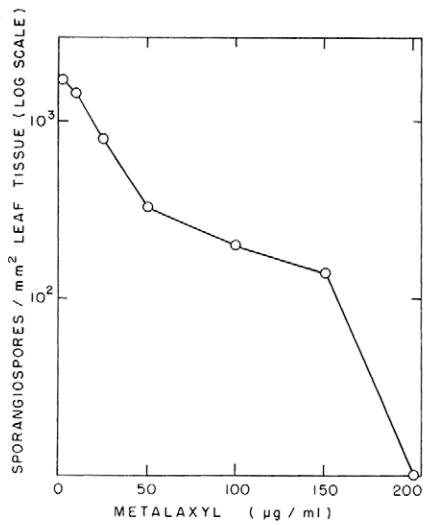

Fig. 1. Inhibition of sporangiospore formation on pea plants infected with *Peronospora viciae* in a closed container. Volatiles emanated from a reservoir of metalaxyl solution.

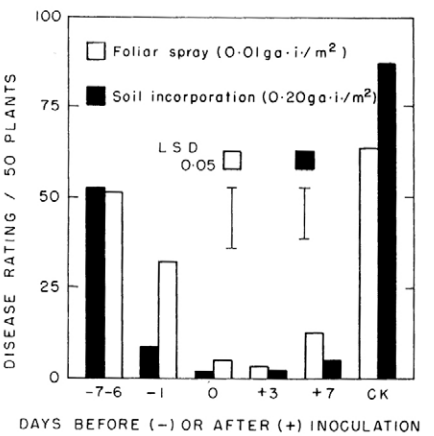

Fig. 2. Comparison of foliar spray and soil incorporation of metalaxyl for control of pea downy mildew caused by *Peronospora viciae*. Controls were not treated with fungicide. Fifty plants per plot were rated with a scale of 0 = symptomless to 4 = abundant sporulation.

Field experiments. Both protective and curative foliar sprays reduced disease compared with the unsprayed control, although plants given a protective spray had higher disease ratings than plants given a curative spray (Fig. 2). Plants sprayed at the first leaf stage (6 days before inoculation) had greater sporulation than plants sprayed 1 day before inoculation. Metalaxyl was most effective when applied at inoculation or 3 days after inoculation. However, control of downy mildew was better on plants sprayed 7 days after inoculation than on plants sprayed 1 or 6 days before inoculation.

As with foliar treatments, metalaxyl was more effective when applied as a curative treatment to the soil than as a protective treatment. Soil application attempted at the first leaf stage (7 days before inoculation), had higher disease ratings on plants than the treatments applied 1 day before inoculation. Disease ratings were again lowest when the fungicide was applied at the time of inoculation or 3 days later.

DISCUSSION

Although systemic fungicides had little success against downy mildews in the past, metalaxyl offers good control of many of these diseases (6,9,11). Efficiency of metalaxyl, as a protective or curative treatment of *P. viciae*, depends on the time of application before or after inoculation. Efficiency declines while metalaxyl is in the plant. Soil applications resulted in better control than foliar sprays, which may have been because metalaxyl was used in larger quantities in soil treatments and fungicide was available longer in the soil. Staub et al (10) reported that metalaxyl is easily taken up by roots of tomato and grape seedlings, transported into the leaves, and penetrates the leaves after foliar spray.

Laboratory studies showed that metalaxyl retards colonization of the host tissues by the fungus. Haustoria were abnormally small and often branched at their distal ends in treated leaves. Metalaxyl also reduced the size of haustoria of *Plasmopora viticola* (9). High concentrations of metalaxyl resulted in vapor concentrations that inhibited sporulation of *P. viciae*. No other reports concerning this gaseous effect have appeared.

Metalaxyl was not phytotoxic to pea in any of our experiments, but Worden (12) reported that concentrations >1.0 g/ml were phytotoxic to tobacco seedlings when applied as a transparent dip against blue mold.

A foliar spray containing 100 g (a.i.) of metalaxyl in 500 L of water per hectare would have a marked curative effect on recently extablished *P. viciae* infections. The time that protection would last is unknown. Slightly more prolonged protection would be achieved by applying 8.0 kg of metalaxyl per hectare to soil before planting, but this would be several times more expensive than foliar sprays.

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