Control of Pythium Blight of Snap Beans by Seed Treatment with Systemic Fungicides

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ABSTRACT

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The systemic fungicide metalaxyl applied to snap bean (*Phaseolus vulgaris*) seed directly or with acetone infusion significantly reduced blight in the greenhouse and field in soils naturally infested with *Pythium ultimum, P. aphanidermatum*, and *P. myriotylum*. Directly applying as little as 0.2 g a.i./kg of seed using either a 50WP or 2E formulation controlled preemergence damping-off and postemergence blight without phytotoxicity. Efficacy of metalaxyl against blight was reduced during incubation at a high temperature (35 C). Metalaxyl and propamocarb hydrochloride seed treatments generally gave better protection than ethazol against Pythium blight. In liquid culture, metalaxyl had an ED_{50} of <1 μg a.i./ml in inhibiting mycelial growth of the three *Pythium* species, with *P. ultimum* the most sensitive. Zoospore and oospore germination and sporangial formation were less sensitive to metalaxyl than mycelial growth. Metalaxyl did not affect membrane permeability at 50 μg/ml, and cholesterol had no effect on leakage.

Additional key words: organic solvent infusion technique

Pythium blight of snap bean (*Phaseolus vulgaris* L.) on the eastern shore of Maryland and elsewhere is caused by any one or a combination of *Pythium aphanidermatum* (Edson) Waterhouse, *P. myriotylum* Drechs., or *P. ultimum* Trow (6,7). This disease, which is important in bean production, can cause losses of 30–100% under favorable environmental conditions (7). Although some resistance to *P. ultimum* has been reported (2), no cultivars resistant to this combination of *Pythium* spp. are available (3,4). In addition, no adequate biological or cultural control measures have been reported. Recently, Papavizas et al (12) reported successful use of some new experimental systemic fungicides as seed treatments in the greenhouse and field to control Pythium blight.

This study reports data on the efficacy of the systemic fungicide metalaxyl as well as the fungicides ethazol and propamocarb hydrochloride as seed treatments for snap beans against damping-off and blight caused by a combination of *Pythium* species. In addition, data presented may explain the mechanism by which metalaxyl is inhibitory to *Pythium* spp.

MATERIALS AND METHODS

Fungicides. The following fungicides were used in the greenhouse and for subsequent field evaluation: metalaxyl (Ridomil 2E or 50WP), ethazol, and propamocarb hydrochloride. Fungicides were applied to seed directly or with a solvent (water or acetone). With direct application, formulations containing liquid fungicides ethazol, propamocarb hydrochloride (70% aqueous solution), and metalaxyl (2E) were added to 1-kg batches of seed at the concentrations indicated in each experiment and the seeds were shaken thoroughly for 10 min and allowed to dry before planting. Metalaxyl 50WP was mixed with graphite and applied to slightly moist seed (1 g seed:2 mg graphite). With the organic solvent infusion technique (11,12), fungicides were dissolved in acetone at concentrations indicated in each experiment and 1-kg batches of seed were immersed in the solutions for 45 min. Acetone was evaporated in a hood and seeds were stored dry until planted. In some experiments, propamocarb hydrochloride was also dissolved in water and infused into seed. In laboratory experiments, metalaxyl 50WP was dissolved in sterile distilled water and added in appropriate amounts (w/v) to autoclaved media before dispensing into flasks or petri dishes.

Greenhouse tests. A loamy sand (pH 6.1) from Salisbury, MD, naturally infested with *P. aphanidermatum, P. myriotylum*, and *P. ultimum* was used for all greenhouse studies (7). Snap bean seed (cultivar Early Gallatin and Bush Blue Lake 274) were planted at a rate of 10 seed/2-L stainless steel beaker containing 1.5 kg of soil. Beakers were incubated in constant-temperature baths at 21 ± 1 C for 1 wk, after which percent emergence was determined. The temperature in the tanks was then raised to 32 C, or as otherwise noted in individual experiments, and the tanks were covered with polyethylene film to increase the relative humidity for an additional week. The beakers were then removed from the tanks and placed in a greenhouse cubicle at 29 ± 3 C. Final plant stand and Pythium blight data were calculated 3 wk after planting. Five replicates were made of each treatment and experiments were repeated at least once.

Field tests. Treated and untreated Bush Blue Lake 274 seeds were planted both in May and July of 1977 and 1978 and in May of 1979 in a field at Salisbury, MD, heavily infested with *Pythium* spp. (7). This field was maintained according to commercial production practices. Seeds were planted in rows 6.1 m long and 90 cm apart at a rate of 100 seeds per row. All treatments were replicated five times. For direct fungicide application, propamocarb hydrochloride, metalaxyl 2E, and metalaxyl 50WP were used to provide 2.2, 0.4, and 0.4 g a.i./kg seed, respectively. Plant stand was determined 3 and 9 wk after planting. At 9 wk, plants were harvested and Pythium blight severity and vine and pod weights determined.

Laboratory tests. Fungitoxicity of metalaxyl to the three *Pythium* spp. associated with bean blight was determined in synthetic basal medium-1 (BM-1) (9). The pH of BM-1 was adjusted to 6.9–7.0 with phosphate buffer. Growth was determined on both liquid (40 ml) and solid BM-1 supplemented with metalaxyl at 0.0, 0.1, 0.25, 0.5, 1.0, 5.0, and 50.0 μg a.i./ml. Inocula consisted of

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5-mm-diameter disks from 7-day-old *Pythium* cultures grown on unamended BM-1. Colony diameters on solid media were measured after 2 and 3 days and mycelium dry weights of flask cultures were determined after 14 days of incubation at 20 C. The ED_{50} values were calculated from the percent inhibition of growth in treatments compared with the control.

Oospores of *P. aphanidermatum* (ATCC 26081) for spore germination tests with metalaxyl were produced aseptically by blending mycelium in 100 ml of V-8 juice-cholesterol medium (1) and incubating the mixture for 2 wk at 23 ± 2 C. Mycelial mats were washed in tap water and rapidly dried in glass dishes. Oospores were collected by blending the dry mats in 25 ml of water and filtering the spores through lens paper, then concentrating them on a nylon mesh screen with 25-μm openings. Droplets of equal volume of oospore suspension (in Czapek nutrient medium with 2% yeast extract) and metalaxyl solution were combined on the surface of a plastic petri dish and germination was determined microscopically after incubation in a humid environment at 30 C for 20 hr.

Zoospores of *P. ultimum* var. *sporangiferum* (ATCC 13647) and *P. aphanidermatum* were produced from mycelial mats grown in the V-8 juice-cholesterol medium for 3–4 days (before oospore formation). Mats were washed in several changes of sterile distilled water and incubated at 23 ± 2 C overnight. Zoospores released from sporangia were used in germination studies similar to those with oospores except incubation was 4.5 hr.

We determined the effect of metalaxyl on production of sporangia of *P. ultimum* var. *sporangiferum* in vitro by placing washed mycelial mats in a solution of soil extract with metalaxyl and incubating them at 23 ± 2 C for 24 hr.

The effect of metalaxyl (50 μg/ml) on leakage of constituents from mycelium of *P. myriotylum* (ATCC 26082) and *P. aphanidermatum* (ATCC 26081) and the possibility of reversal by sterol was tested by methods previously described for other fungicides (14). Soluble carbohydrate was determined as anthrone-positive material (8) and total soluble inorganic salts with a conductivity bridge (5). All laboratory experiments were performed three times in triplicate.

RESULTS

Greenhouse tests. Direct application of either 50WP or 2E formulations of metalaxyl as low as 0.2 g a.i./kg of seed (Early Gallatin) significantly increased plant stand and reduced Pythium blight compared with the control (Table 1). Plant stand was increased up to 67% and blight was reduced up to 92% with 0.2 g metalaxyl per kilogram of seed. Disease reduction was similar regardless of fungicide formulation; higher amounts of metalaxyl were not more effective than the lowest amount used. Treatment with fungicide up to 1.4 g/kg of seed was not phytotoxic to this cultivar under the conditions tested.

An experiment was performed to verify these results with another bean cultivar (cultivar Bush Blue Lake 274) and to determine the influence of temperature on the efficacy of metalaxyl applied directly and by acetone infusion to seed. All treatments significantly increased plant stand over the untreated control 1 wk after planting and before incubating the seedlings at 29, 33, or 35 C. The stand from untreated seed was 28%, whereas that from seed treated directly with metalaxyl at 0.2, 0.4, and 0.8 g/kg seed was 86, 86, and 84%, respectively. The stand from seed treated by acetone infusion was 84%. Pythium blight control, however, was dependent on temperature (Fig. 1). All treatments gave significant control at temperatures of 29 and 33 C, but at incubation of 35 C, only the highest rate (0.8 g/kg seed) of directly applied metalaxyl gave significant protection (31% blight) over the control (100% blight). Although blight control decreased with increasing temperature, it should be noted that disease pressure increased with increasing temperature, especially in the 35 C treatment.

In another experiment, we compared the efficacy of metalaxyl with that of ethazol and propamocarb hydrochloride for reducing damping-off and blight in the greenhouse. In addition, methods of application were compared. All treatments except direct application of ethazol (1.0 g a.i./kg seed) resulted in significantly improved stands and reduced blight over the control (Table 2). Application of propamocarb hydrochloride in water was less effective in reducing blight than direct application or acetone infusion.

Field tests. The wide variability in the plant stands of control plots from five plantings over a 3-yr period (Table 3) reflect the influence of climatic and ecological factors on Pythium blight of beans. Disease was most severe, for example, during the latter part of the planting season in 1977 and throughout 1978, whereas minimal blight occurred during the first planting of 1979. In our field experiments over a 9-wk period, blight was directly related to plant stand; blighted plants by this time were not harvestable. Formulations of propamocarb hydrochloride, ethazol, and metalaxyl were applied to seed by either of two methods during the five plantings within the 3-yr period.

Direct application of propamocarb hydrochloride did not effectively reduce blight in the three plantings when it was used, but infusion of seed with two concentrations of the material did reduce

Table 1. Effect of metalaxyl applied to snap bean seed (cultivar Early Gallatin) on damping-off and blight caused by *Pythium* spp. in the greenhouse[x]

Metalaxyl concentration (g a.i./kg seed)	Plant stand (%) with indicated formulation[y]		Pythium blight (%) with indicated formulation[y]	
	50WP	2E	50WP	2E
0	60 a[z]	54 a	100 a	48 a
0.2	96 b	80 b	25 b	4 b
0.4	94 b	92 b	22 b	2 b
1.4	94 b	90 b	0 b	0 b

[x] Metalaxyl 2E applied to seed directly; 50WP mixed with graphite before application (1 g seed:2 mg graphite).
[y] At 3 wk after planting.
[z] The experiments with the two formulations were not done concurrently. Numbers in each column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

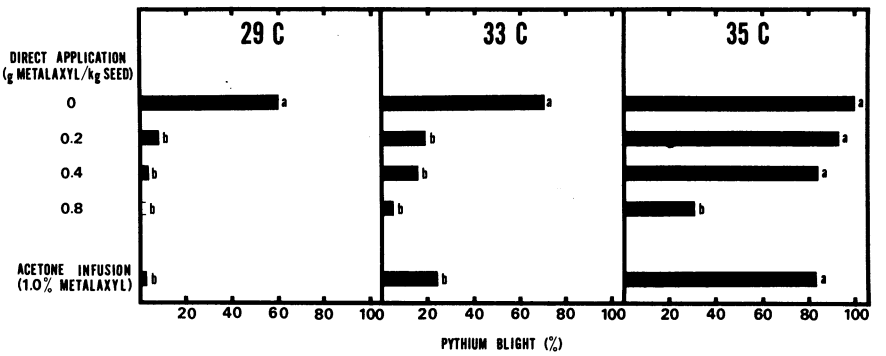

Fig. 1. Influence of temperature on efficacy of metalaxyl seed treatment for Pythium blight of snap bean (cultivar Bush Blue Lake 274) in the greenhouse. Blight expressed as percentage of original stand was determined after incubation for 1 wk at 21 C followed by 2 wk at indicated temperature. Bars at each temperature followed by same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

blight in one of three plantings. Seed infused with ethazol was planted on all five planting dates, but only in the first planting of 1978 did the chemical, at the low rate, significantly reduce blight. Contrary to this, ethazol was phytotoxic, probably because of climatic conditions, during the first planting of 1979 because emergence was low at the beginning of the experiment. Seed treated directly with metalaxyl was planted in 1977, 1978, and 1979 and with metalaxyl by infusion in 1978. Metalaxyl 2E applied directly significantly reduced blight in two of three trials, whereas metalaxyl 50WP was ineffective. Metalaxyl applied in two concentrations to seed by infusion effectively reduced blight in the first planting but not under the severe blight pressure of the second planting.

Table 2. Effect of fungicides applied to snap bean seed (cultivar Bush Blue Lake 274) directly or with the organic solvent infusion technique on damping-off and blight caused by *Pythium* spp. in the greenhouse

Fungicide and concentration	Immersion time (min)	Plant stand[y] (%)	Pythium blight[y] (%)
Direct fungicide application			
None (control)	...	30 c[z]	93 a
Ethazol (1.0 g a.i./kg seed)	...	32 c	94 a
Propamocarb hydrochloride (5.6 g a.i./kg seed)	...	78 ab	8 c
Metalaxyl (0.4 g a.i./kg seed)	...	78 ab	16 c
Solvent infusion technique			
Acetone (control)	...	34 c	100 a
Ethazol 5% in acetone	60	76 ab	9 c
10% in acetone	60	74 ab	3 c
Propamocarb hydrochloride 2.5% in water	15	78 ab	48 b
2.5% in water	30	90 a	34 b
2.5% in acetone	60	76 a	16 c
2.5% in acetone	120	60 b	23 c
Metalaxyl 1% in acetone	45	80 ab	14 c

[y] At 3 wk after planting.
[z] Numbers in each column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Effect of fungicides applied to snap bean seed (cultivar Bush Blue Lake 274) directly or with the acetone infusion technique on Pythium blight in the field during 1977, 1978, and 1979

	Plant stand (%)[y]				
	1977		1978		1979
Fungicide[x]	First planting	Second planting	First planting	Second planting	First planting
Direct application					
None (control)	49 a[z]	15 a	17 bc	16 bc	88 a
Propamocarb hydrochloride	43 a	25 a	46 bc	...	...
Metalaxyl 2E	...	...	63 a	26 a	84 a
Metalaxyl 50WP	41 a	...	...	21 abc	...
Acetone infusion					
Acetone (control)	43 a	12 a	14 c	12 c	81 a
Ethazol 5%	47 a	12 a	62 a	13 c	52 b
Ethazol 10%	47 a	10 a	46 bc	11 c	33 c
Propamocarb hydrochloride 2.5%	43 a	22 a	63 a	...	...
Propamocarb hydrochloride 5%	...	...	53 ab	...	...
Metalaxyl 0.75%	...	...	71 a	23 ab	...
Metalaxyl 1.5%	...	...	62 a	...	...

[x] For direct application, formulations of propamocarb hydrochloride, metalaxyl 2E and 50WP were used to provide 2.2, 0.4, and 0.4 g a.i./kg seed, respectively. For acetone infusion, immersion time was 45 min.
[y] At harvest, 9 wk after planting.
[z] Numbers in each column followed by same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Table 4. Effect of metalaxyl on zoospore and oospore germination and sporangia formation in two species of *Pythium*[u]

Metalaxyl concentration (μg/ml)	Germination (%)[v]			Sporangia (no./field)[x]
	Zoospores		Oospores	
	Pu[w]	Pa	Pa	Pu
0	95 a[y]	100 a	42 a	23 a
0.25	...[z]	99 a	24 c	...
1.0	80 b	98 a	30 b	...
10.0	82 b	100 a	25 bc	11 b
50.0	69 c	100 a	20 c	12 b

[u] Spore germination determined in Czapek broth supplemented with 2% yeast extract and sporangial formation in soil extract.
[v] Germination recorded after 4.5-hr incubation for zoospores and 20 hr for oospores.
[w] Pu = *Pythium ultimum* var. *sporangiferum*; Pa = *Pythium aphanidermatum*.
[x] Sporangia counted per ×90 microscope objective field after 24 hr in unsterile soil extract.
[y] Numbers in each column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[z] Combination not included in the experimental design.

At harvests, vine and pod weight from all treatments were variable. Although vine weight from metalaxyl and ethazol treatments tended to be more than that of the control, there were no significant correlations between vine weight increase and blight reduction. Beneficial seed treatments, especially those with metalaxyl, in many cases resulted in yield increase. For example, during the first planting of 1978, yield was 1.8 and 1.5 kg of pod per row from directly applied and infusion-applied metalaxyl, respectively. These values were significantly greater ($P = 0.05$) than the 1.2-kg yield from the control.

Laboratory tests. Significant disease reduction in the greenhouse and field resulting from application of metalaxyl to seed prompted us to examine the inhibitory effect of the chemical directly on mycelial growth, sporangia formation, and spore germination. The mycelial growth ED_{50} values for metalaxyl against *P. ultimum, P. aphanidermatum*, and *P. myriotylum* were <0.1, 0.25–0.5, and 0.5–1.0 μg a.i./ml, respectively, in liquid culture when measurement was made after 14 days of incubation. On solid BM-1, ED_{50} values were higher than those obtained in liquid culture. Inhibition was no longer apparent after 21 days of incubation of cultures in liquid or solid media. *P. ultimum* was the most sensitive of the three species to metalaxyl.

A relatively high concentration of metalaxyl was necessary to reduce in vitro zoospore germination of *P. ultimum* because less than 50% reduction occurred with as much as 50 μg of metalaxyl per milliliter (Table 4). Zoospore germination of *P. aphanidermatum* however, was not inhibited at all with this concentration. Oospore germination of *P. aphanidermatum* was reduced by 43% at 0.25 μg/ml, but larger amounts of the fungicide did not increase the inhibition. Metalaxyl also reduced sporangia formation in *P. ultimum* (Table 4). In the absence of the fungicide, 22 sporangia per microscope field were formed after 24 hr in unsterile soil extract, whereas 11 and 14 were formed with 10 and 50 μg/ml of metalaxyl, respectively.

The effect of metalaxyl on membrane permeability was studied using mycelium of *P. myriotylum*. Determination of

soluble carbohydrates and soluble salts leaked from fungus mycelium into distilled water indicated that metalaxyl did not cause membrane damage. Thirty-three and 30 mg of anthrone-positive materials per gram mycelial dry weight were leaked by control mats and mats suspended in metalaxyl, respectively. Conductivity readings of salts in solution from these mats were 255 and 293 μmho/g mycelial dry weight, respectively. Cholesterol in the growth medium did not influence metalaxyl in increasing or decreasing leakage. Similar results were obtained using mycelium of *P. aphanidermatum*.

DISCUSSION

The potential for metalaxyl as an effective seed treatment for reducing Pythium blight of snap beans is demonstrated by the greenhouse and field data presented in this paper. This expectation is also confirmed by recent research that showed that small amounts of the fungicide applied to cottonseed (*Gossypium hirsutum* L.) completely prevented damping-off caused by a mixture of *Pythium* spp. (13). Little work has been reported on the efficacy of various fungicides for controlling Pythium blight of bean. Registered fungicides do not control the disease, and ethazol, which has been used as a protectant for Pythium blight of bentgrass (*Agrostis palustris* Huds.) (15), was not as effective as metalaxyl in controlling bean blight in our greenhouse and field experiments. Our results also demonstrate that the solvent infusion technique appears better than direct application of fungicides for disease control. Both propamocarb hydrochloride and metalaxyl were more effective in the field when applied to seed by infusion rather than directly. This observation is consistent with an earlier one (12) in which ethazol and propamocarb hydrochloride were more effective for blight control when they were applied to seed by infusion than by direct application.

Laboratory results, which demonstrated that metalaxyl was more toxic to *P. ultimum* than to the other two species, may explain why metalaxyl was unable to suppress disease at a high temperature (35 C) when applied at low or medium rates. At 35 C, the increase in disease pressure may have been due to the activity of *P. aphanidermatum* and *P. myriotylum*, both of which favor high temperature and are less sensitive to the fungicide than *P. ultimum*. Also, snap bean susceptibility to the disease may increase at high temperatures or metalaxyl may break down at such temperatures. The adverse effect of high temperatures on seed treatments was also demonstrated in the field, where ethazol showed toxicity during May and June 1979, when soil temperatures for that year at Salisbury were exceptionally high.

The low ED_{50} value (<1.0 μg a.i.) of metalaxyl against the three species of *Pythium*, with *P. ultimum* the most sensitive to the fungicide, further indicates that metalaxyl has the potential of becoming a very useful fungicide for Pythium control, especially for *P. ultimum*, which causes seed rots and preemergence damping-off. Activity of metalaxyl against *Pythium* spp. is similar to that observed recently with various isolates of *Phytophthora capsici* Leonian (10). Metalaxyl at low concentrations significantly reduced oospore germination and sporangial formation in *P. capsici*. We observed similar reactions to metalaxyl with *Pythium* spp. Metalaxyl did not increase membrane permeability of mycelium of *Pythium* spp. as has been reported for the fungicide propamocarb hydrochloride (14); consequently, the mode of action of metalaxyl is apparently not related to alteration of membrane permeability.

Our findings indicate that seed treatment with the systemic fungicide metalaxyl has potential for reducing the devastating effect of Pythium blight, but further development of application methods is necessary to achieve acceptable yield returns.

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