Chemical Control of Stem and Root Rot of Cowpea Caused by *Phytophthora vignae*

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ABSTRACT

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The fungicides metalaxyl (Subdue), fosetyl-Al (Aliette 80W), etridiazole (Banrot), and mancozeb (Manzate 200) significantly reduced in vitro mycelial growth of *Phytophthora vignae* isolates P001 and P006 at most concentrations tested. Metalaxyl was the most effective compound at low concentrations in both in vitro and pot experiments in reducing mycelial growth (1.68 mg a.i./L and 2.45 mg a.i./L for P001 and P006, respectively), oogonia formation (1.03 and 1.07 mg a.i./L for P001 and P006, respectively), and disease severity (10 mg a.i./L), and in increasing plant dry weight (10 mg a.i./L). High concentrations of metalaxyl (50 and 100 mg a.i./L) were phytotoxic and reduced plant and root dry weights. In contrast, fosetyl-Al was effective only at high concentrations. Mancozeb was effective both as a foliar spray and as a soil drench. Etridiazole was effective at low concentrations (5.75 mg a.i./L) in reducing radial growth of the fungus and decreased disease severity and increased plant dry weight at 50 mg a.i./L. Growth in vitro of strain DF-3101 of the biocontrol bacterium *Brevibacterium linens* was not affected by metalaxyl and etridiazole, but growth was partially inhibited by mancozeb and completely inhibited by 1,250 mg a.i./L of fosetyl-Al. Capacity for production of diffusible inhibitors by strain DF-3101 was not decreased significantly by prior exposure to 5 mg a.i./L of metalaxyl or 10 mg a.i./L of etridiazole but was decreased by 10 mg a.i./L of metalaxyl and 50 mg a.i./L of mancozeb on potato-dextrose agar; production of volatile inhibitors was not affected significantly by any chemical. All four fungicides tested were effective against *P. vignae* and could be used alternatively to reduce the development of fungicide resistance. Furthermore, all fungicides except fosetyl-Al could be applied at low concentrations in combination with bacterial biocontrol agents.

Additional keyword: toxicity

Stem and root rot of cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*) caused by *Phytophthora vignae* Purss is one of the most serious soilborne diseases of cowpea in Australia (24) and Japan (21). It has also been reported in India (22), Taiwan (19), South Korea (16), and, most recently, Sri Lanka (13,28). Chemical control strategies for the pathogen on cowpea have not been described. Systemic fungicides for the control of soilborne diseases caused by fungi of the order Peronosporales were first developed in the early 1970s (26). Metalaxyl, an acylalanine, and fosetyl-Al, an ethyl phosphite, are systemic fungicides now used against a variety of *Phytophthora* species causing diseases of different crops (1,8,23,31).

The objectives of this study were to evaluate the efficacy of four fungicides in inhibiting *P. vignae* and cowpea stem and root rot and to determine their compatibility with the bacterial biocontrol agent *Brevibacterium linens* (Wolff) Breed.

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MATERIALS AND METHODS

Pathogen and fungicides. *P. vignae* isolates P001 and P006, obtained from cowpea fields of Sri Lanka, were used in these experiments. We (13) placed isolates P001 and P006 in two distinct groups, based on average cluster analysis. Isolate P001 was used in all experiments because it is the more commonly encountered race, and P006 was used only in culture experiments.

The fungicides used were metalaxyl 2EC (Subdue), fosetyl-Al (Aliette 80W), mancozeb (Manzate 200), and etridiazole (Banrot, etridiazole + thiophanate-methyl). Stock solutions or suspensions were prepared by mixing each fungicide in sterile distilled water. These solutions then were used for in vitro tests as amendments to potato-dextrose agar (PDA) or in pot experiments as soil drenches or foliar sprays. All concentrations are given as active ingredients (a.i.).

Mycelial growth in fungicide-amended media. Mycelial growth was measured on PDA plus an additional 1% agar amended with different concentrations of the four fungicides. The fungicides were filter-sterilized after stock solutions were prepared. The PDA was autoclaved and cooled to 45 C before fungicide solutions were added. A graduated sterile syringe apparatus was used to add 20 ml of molten agar medium to each 100 × 15 mm plastic petri plate. Isolates P001 and P006 were grown for 7 days at 25 C in darkness on PDA amended with 20 mg/L of pimaricin. Then, 7-mm-diameter plugs were cut from actively growing colony margins and placed in the center of fungicide-amended medium in five replicate plates per treatment. PDA was amended with 0, 1, 5, and 10 mg a.i./L of metalaxyl; 0, 500, 750, 1,000, and 1,250 mg a.i./L of fosetyl-Al; 0, 1, 5, 10, and 50 mg a.i./L of etridiazole; and 0, 1, 5, 10, and 50 mg a.i./L of mancozeb. In initial experiments with 1, 5, and 10 mg a.i./L of fosetyl-Al, the fungus grew as much on the amended plates as on the control plate, so these concentrations were not included in the main experiment. Inoculated plates were incubated in a cabinet at room temperature (25 C) in darkness for 8 days. Mycelial growth (colony diameter) was measured daily.

The experiment was a completely randomized design regarding plate placement, with five replicates, and was repeated once.

Inhibition of oogonia formation. Inhibition of oogonia formation was determined on cowpea extract agar (CPA; crude extract of 200 g of cowpea stems and roots plus 20 g of agar in 1,000 ml of distilled water) amended with 0, 1, 5, 10, 50, and 100 mg a.i./L of metalaxyl or 0, 10, 50, 100, and 1,000 mg a.i./L of fosetyl-Al. Fungicides were added to the CPA medium first cooled to 45 C. A 7-mm plug of isolate P001 or P006 was placed in each of three replicate plates per treatment. Plates were incubated at 25 C in darkness for 2 wk in a completely randomized design. Five random sample areas around the initial plug in each of the three plates (a total of 15 areas) were selected for counting oogonia with a light microscope at 100×. The experiment was repeated twice.

Efficacy of fungicides in greenhouse pot experiments. Inoculum was prepared by amending 500 ml of coarse vermiculite in flasks with 300 ml of V8 juice solution (200 ml of V8 concentrate, 800 ml of distilled water, and 2 g of $CaCO_3$) and autoclaving the mixture for 1 hr and again 24 hr later for 20 min. Isolate P001 was grown on PDA amended with 20 mg/L of pimaricin for 1 wk before 10 7-mm-diameter plugs were transferred to each vermiculite flask. Inoculated flasks were incubated at 25 C in darkness for 1 mo.

Seeds of susceptible cowpea cv. California Blackeye were planted in a pasteurized (air-steamed at 60 C for 60 min) sand-soil mix (50:50, v/v) in 10-cm-diameter plastic pots. Plants were grown on greenhouse benches for 3 wk under a 16-hr light period with supplementary light (350–480 $\mu E \cdot m^{-2} \cdot s^{-1}$) from high-pressure sodium vapor lamps. The first application of fungicides was made 22 days after sowing. There were 12 treatments: metalaxyl at 10, 50, and 100 mg a.i./L as a soil drench; fosetyl-Al at 1,000 and 2,000 mg a.i./L as a foliar spray; mancozeb at 50 and 100 mg a.i./L as a foliar spray and 100 mg a.i./L as a soil drench; etridiazole at 10 and 50 mg a.i./L as a soil drench; and two controls (plants inoculated or not inoculated with the pathogen). Soil drenches were applied to the surface, 100 ml per pot. Foliar sprays were applied to leaves until runoff. Before spraying, 100 ml of water was added to the soil; during spraying and for 6 hr after, the soil surface was covered with brown paper to prevent fungicide contamination of the soil. On day 30 after sowing, each plant was removed carefully from the pot, pathogen inoculum was mixed into the soil, and the plant was repotted. The noninoculated control treatment was amended with moistened sterile vermiculite-V8 medium at the same volume. Fungicides were applied again 7 days after inoculation. Plants were watered every other day and fertilized once a week with Long Ashton Nutrient Solution (17). Plant treatments were arranged on the greenhouse bench in a completely randomized design, with 10 replicates of each treatment.

Disease severity was rated 2 wk after inoculation. On the 0–5 scale for stems, 0 = no disease symptoms; 1 = leaves turning pale green, wilting, and/or stem lesions appearing on the stem base; 2 = leaves severely wilting and stem lesions advancing upward; 3 = symptoms advancing plus pronounced drying and stem lesions spreading over 2 cm; 4 = 70–90% of plant showing symptoms with or without stem girdling; and 5 = plant dead. On the 0–4 scale for roots, 0 = no disease symptoms, 1 = <25% of lateral roots with brown lesions but no taproot necrosis, 2 = 25–50% lateral root necrosis and <50% taproot infection, 3 = 50–75% lateral root necrosis and 50–75% taproot necrosis, and 4 = >75% of lateral root and taproot necrosis. Stems and roots were oven-dried for 72 hr at 65 C and their dry weights determined. The experiment was repeated once.

Responses of biocontrol on fungicide-amended media. This experiment was designed to determine if exposure to efficacious levels of fungicides would affect the antagonistic potential of a biocontrol bacterium. The concentrations of fungicides that fully inhibited in vitro growth of isolate P001 in PDA were used: metalaxyl at 5 and 10 mg a.i./L, fosetyl-Al at 1,250 mg a.i./L, etridiazole at 10 mg a.i./L, and mancozeb at 50 mg a.i./L.

B. linens strain DF-3101 isolated from a cowpea field that harbored the pathogen but showed no apparent disease on plants and effectively suppressed stem and root rot on cowpea (13) was used. Bacteria were cultured in tryptic soy broth for 48 hr at 30 C on a rotary shaker at 150 rpm and then transferred onto fungicide-amended PDA plates with 20 ml of medium as a loop streaked zigzag exactly the same number of times on each plate. The control plates contained PDA without fungicides. Bacteria were incubated at 27 C for 2 wk before viability and capacity to produce inhibitors of *P. vignae* were determined. The experiment was a completely randomized design with five replicates per treatment and was repeated once. Two weeks later, bacteria from each fungicide-treated and control plate were streaked onto one half of divided petri plates containing tryptic soy agar (TSA), King's medium B (KMB) (20), or nutrient agar (NA). The other side of the divided plates contained PDA. Three replicate plates for each treatment were incubated for 4 days at 27 C. Then, 7-mm-diameter PDA plugs of *P. vignae* isolate P001 were transferred onto the side of the plates containing PDA, the plates were incubated for 5 days, and mycelial growth was measured as an indicator of response to volatile inhibitors produced by the bacteria. Bacterial streaks were also made in the middle of PDA and TSA whole plates, incubated for 5 days at 27 C, and challenged with isolate P001. The zone of inhibition from diffusible inhibitors was measured.

The amount of plate covered (growth) by bacteria on fungicide-amended media was rated on a scale of 0–4 in which 0 = no growth, 1 = <50%, 2 = 50–75%, 3 = 75-90%, and 4 = 100% of zigzag streaks covered with bacterial growth. Ratings were made after 2 wk of incubation of three replicate plates. The experiment was a completely randomized design and repeated once.

Statistical analysis. Regression analysis was performed on the log of fungicide concentration, and the dependent variables tested were mycelial growth or oogonial inhibition (27). Effects of fungicide concentrations on the disease severity on roots and stems and the shoot and root dry weights were analyzed. The antibiotic and volatile activity of the biocontrol bacterium in the presence of different fungicide concentrations were analyzed by mean separation. Means were separated using the Waller-Duncan k-ratio test at $P < 0.05$ (27).

RESULTS

Mycelial growth in fungicide-amended media. All of the four fungicides tested inhibited growth of *P. vignae*. The lowest fungicide concentration required for 50% inhibition (EC_{50}) of mycelial growth on both isolates of *P. vignae* was with metalaxyl; the EC_{50} for fosetyl-Al was much higher. The concentration of etridiazole needed for 50% inhibition of *P. vignae* radial growth was about 5 μg/ml, regardless of the isolate. Isolate P001 was more sensitive than P006 to mancozeb (Table 1).

Inhibition of oogonia formation. Metalaxyl was more effective than fosetyl-Al at inhibiting oogonia formation. The EC_{50} values for metalaxyl were 1.03 and 1.07 for P001 and P006, respectively, whereas the values for fosetyl-Al were 72 mg a.i./L for P006 and >100 mg a.i./L but <1,000 mg a.i./L for P001, which inhibited oogonial formation completely.

Efficacy of fungicides in greenhouse pot experiments. Metalaxyl drenches provided the best disease control, even at the lowest concentration, 10 mg a.i./L (Table 2). Metalaxyl-treated plants showed no aboveground disease symptoms. The shoot dry weight was similar with 10 mg a.i./L of metalaxyl and the noninoculated control, but root dry weight was greater with the fungicide treatment. The greatest reduction in root disease severity was on plants treated with 100 mg a.i./L of metalaxyl (Table 2).

Among the foliar sprays, 1,000 mg a.i./L of fosetyl-Al and 50 and 100 mg a.i./L of mancozeb reduced disease severity on stems similarly. Doubling the concentration of fosetyl-Al decreased disease severity on stems and roots twofold (Table 2). Mancozeb was more effective in reducing stem and root disease severity as a soil drench at 100 mg a.i./L than as a foliar spray at the same concentration.

Etridiazole applied as a soil drench was not very effective at controlling disease at concentrations of 10 mg a.i./L but was as effective at 50 mg/L as metalaxyl at 10 mg/L in protecting against the fungus and in increasing shoot and root dry weights (Table 2).

Responses of biocontrol on fungicide-amended media. *B. linens* strain DF-3101, which was very effective in biological control experiments (14), did not grow on PDA media amended with 1,250 mg a.i./L of fosetyl-Al, and growth was reduced by 75% when the medium was amended with 50 mg a.i./L of mancozeb. In all other treatments, bacterial growth was similar to that of the nonamended control.

In the test for retention of antagonistic capacity following growth on fungicide-amended media for 2 wk, the potential for bacterial antagonism was decreased significantly ($P = 0.05$) when grown on PDA following treatment with 10 (but not 5) mg a.i./L of metalaxyl and 50 mg a.i./L of mancozeb (Table 3). Antagonistic activity after growth on fosetyl-Al could not be tested because the bacterium did not grow on amended media. On TSA, the bacterium retained its inhibitory capacity, and inhibition was equal to that of the control plate in all treatments (Table 3).

In most cases, the pathogen grown on PDA was completely inhibited by the bacterium grown on TSA. Strain DF-3101 grown on KMB or NA amended with 50 mg a.i./L of mancozeb allowed some growth of the pathogen on PDA, but growth was not significantly different from that on the control. Strain DF-3101 therefore was fully capable of producing volatile inhibitors and was not significantly different from the control (Table 3). Because 1,250 mg a.i./L of fosetyl-Al completely inhibited the bacterium, no further tests were possible.

DISCUSSION

Of the four fungicides tested in vitro, metalaxyl was most effective at low concentrations in inhibiting *P. vignae* isolates P001 and P006. Farih et al (9) noted a similar effect, even at a lower concentration of metalaxyl, on *P. citrophthora* and *P. parasitica*. However, noneffectiveness of metalaxyl in media (up to 350 mg a.i./L) was reported by Bruck et al (2), who used amended lima bean agar (LBA). Ellis et al (7) used LBA and found that 1 mg a.i./L of metalaxyl inhibited mycelial growth of *P. cactorum* completely, but when the plug was transferred to LBA without fungicide, the fungus grew, suggesting that metalaxyl was more fungistatic than fungicidal.

Our studies also confirm other reports that fosetyl-Al does not inhibit mycelial growth effectively unless used at high concentrations (11,29,30). Isolate P006 of *P. vignae* was not inhibited completely

Table 1. EC_{50} values for inhibition of radial growth of *Phytophthora vignae* isolates P001 and P006 on PDA amended with four different fungicides

	Radial growth inhibition EC_{50}[a] (μg/ml)			
	Isolate P001		Isolate P006	
Fungicide	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Metalaxyl (Subdue)	1.68[b]	1.73	2.45	2.63
Fosetyl-Al	420.00	420.00	680.00	674.00
Etridiazole (Banrot)	5.75	5.00	5.19	5.35
Mancozeb (Manzate 200)	12.47	13.08	19.32	20.05

[a] EC_{50} is the concentration required to inhibit radial growth of the fungus by 50%. Radial growth was measured after 7 days of incubation at 26 C. The values are means from two experiments. The level of significance of the regressions is $P < 0.01$.
[b] Regression analyses of fungicide concentration vs. mycelial growth were significant. The ranges of concentrations of the fungicidal compounds were: metalaxyl, 1–10 μg/ml; fosetyl-Al, 500–1,250 μg/ml; etridiazole, 1–50 μg/ml; and mancozeb, 1–50 μg/ml.

Table 2. Effects of fungicide application on control of *Phytophthora vignae* root and stem rot of cowpea[a]

Treatment[b]	Stem DSI[c]	Root DSI[d]	Shoot dry weight (g)	Root dry weight (g)
Metalaxyl (Subdue)				
10 μg/ml SD	0.0	0.3 ± 0.15	3.82 ± 0.29	1.32 ± 0.05
50 μg/ml SD	0.0	0.2 ± 0.13	2.79 ± 0.30	1.31 ± 0.14
100 μg/ml SD	0.0	0.1 ± 0.10	2.74 ± 0.27	1.28 ± 0.13
Fosetyl-Al				
1,000 μg/ml F	1.6 ± 0.56	2.6 ± 0.47	2.27 ± 0.37	0.65 ± 0.12
2,000 μg/ml F	0.8 ± 0.46	1.2 ± 0.38	2.89 ± 0.28	0.93 ± 0.11
Mancozeb (Manzate 200)				
50 μg/ml F	1.6 ± 0.58	1.9 ± 0.37	2.95 ± 0.22	0.70 ± 0.04
100 μg/ml F	1.6 ± 0.42	2.5 ± 0.45	2.24 ± 0.21	0.62 ± 0.08
100 μg/ml SD	0.6 ± 0.33	1.2 ± 0.32	3.22 ± 0.26	1.10 ± 0.14
Etridiazole (Banrot)				
10 μg/ml SD	2.5 ± 0.50	3.1 ± 0.40	2.44 ± 0.30	0.60 ± 0.11
50 μg/ml SD	0.4 ± 0.22	1.0 ± 0.39	3.82 ± 0.21	1.32 ± 0.13
Controls				
Pathogen only	3.0 ± 0.53	3.6 ± 0.22	1.52 ± 0.18	0.32 ± 0.05
Noninoculated	0.0	0.3 ± 0.21	3.90 ± 0.16	0.99 ± 0.09

[a] Each value is the mean of 10 replicates ± SE. Analysis of variance indicated significant ($P < 0.01$) effect of fungicides on disease severity and dry weights, except etridiazole at 10 μg/ml. Results from experiment 1 are shown; similar results were obtained in experiment 2.
[b] SD = soil drench, F = foliar sprays.
[c] Stem disease severity index: 0 = no disease to 5 = plant dead.
[d] Root disease severity index: 0 = no disease to 4 = >75% necrosis of lateral root and taproot.

Table 3. Inhibitor-producing (diffusible or volatile) capacity of *Brevibacterium linens* strain DF-3101, previously exposed to fungicides, against isolate P001 of *Phytophthora vignae*[a]

	Diffusible		Volatile	
Pretreatment exposure	PDA	TSA	KMB	NA
Untreated control	10.3	0.0	0.0[b]	0.0
Metalaxyl (Subdue)				
5 mg/L	15.7	0.0	1.7	0.0
10 mg/L	19.7*[c]	0.0	0.0	0.0
Etridiazole (Banrot)				
10 mg/L	16.8	0.0	3.3	0.0
Mancozeb (Manzate 200)				
50 mg/L	19.7*	0.0	4.0	2.7

[a] The experiment was done on potato-dextrose agar (PDA) or tryptic soy agar (TSA) to test for diffusible inhibitors and on King's medium B (KMB), nutrient agar (NA), or TSA to test for volatile inhibitors. Values are mycelial growth in millimeters and are the means of three replicates. Results from experiment 1 are shown; similar results were obtained in experiment 2.
[b] No treatment significantly ($P = 0.05$) reduced the capacity of strain DF-3101 to produce volatile inhibitors when grown on KMB, NA, or TSA. *P. vignae* was grown on the PDA side of divided petri plates.
[c] * = Significantly different from the control at $P < 0.05$, Waller-Duncan k-ratio test.

even at concentrations >1,250 mg a.i./L, whereas P001 was, suggesting that P006 is more tolerant. In contrast, Fenn and Coffey (12) reported mycelial growth inhibition of *P. cinnamomi* with fosetyl-Al at concentrations <200 mg a.i./L in low-phosphate medium.

The effectiveness of etridiazole and mancozeb at relatively low concentrations suggests that they could be used in combination or as application alternations with fungicides such as metalaxyl and fosetyl-Al. This would help reduce the development of fungicide resistance (4,18,25).

Metalaxyl, even at 1 mg a.i./L, effectively reduced oogonia formation by *P. vignae* by 50%. Inhibition by fosetyl-Al differed with the two isolates of the pathogen. Farih et al (10) reported that fosetyl-Al at 125 mg a.i./L effectively reduced oospore formation in *P. parasitica*.

Fungicide treatments to control disease in the greenhouse confirmed the in vitro studies. Metalaxyl most effectively reduced disease severity on stem and roots, even at lower concentrations than the other chemicals, and mean dry weights of shoots and roots also increased. At concentrations of 50 and 100 mg a.i./L, however, phytotoxicity was indicated by reduced root and shoot dry weight, even though disease severity was the lowest. Davis (5) observed phytotoxicity when metalaxyl was applied at 200 mg a.i./L, but not at 100 mg a.i./L, to control *P. parasitica* in citrus. Cowpea roots are probably finer and thus more sensitive than citrus roots. However, we did not observe the phytotoxic symptoms reported by Davis (5).

High concentrations of fosetyl-Al were needed to significantly control disease caused by *P. vignae* in vivo, which confirms the in vitro culture tests and other reports (5,9,12). Fenn and Coffey (12) indicated there was no difference in effectiveness of fosetyl-Al as a foliar spray or a soil drench when applied at a high concentration. There is good evidence that fosetyl-Al is xylem- and phloem-translocated (3). Our results confirm those of Sandler et al (26), who noted that metalaxyl drenches increased feeder root densities, in contrast to fosetyl-Al, as metalaxyl was capable of killing the fungus in soil. Davis (6) recommended multiple applications of fosetyl-Al to maintain activity over a long period of time. The duration of our study did not allow us to test this recommendation, but the two applications we used presumably would be sufficient to control a *Phytophthora* disease in an annual crop such as cowpea.

When applied as a foliar protectant fungicide, mancozeb effectively reduced disease at both 50 and 100 mg a.i./L. There was a reduction in shoot and root dry weights with 100 mg a.i./L compared with 50 mg a.i./L. This could be due partly to phytotoxicity at higher concentrations. Mancozeb at 100 mg a.i./L was effective as a soil drench, as was etridiazole at 50 mg a.i./L. This effectiveness may enable a grower to alternate applications of two chemicals to prevent the buildup of pathogen resistance.

Our results with fungicide treatments on the bacterial biocontrol agent *B. linens* strain DF-3101 suggest that bacterial antagonism through diffusible antibiotic production may be affected by fungicides applied at high concentrations but that production of volatile inhibitors is not. Utkhede (33) reported no inhibition by *Enterobacter aerogenes* strain B-8 antibiotic production or bacterial growth in the presence of the fungicides tested. Also, Gupta and Utkhede (15) tested the effect of bacteria and fungicide combinations in soil and found that multiplication of antagonistic bacteria was enhanced by fosetyl-Al at lower temperatures and by metalaxyl at higher temperatures. Utkhede (32) also reported that metalaxyl + mancozeb allowed significantly more *Phytophthora* infection than metalaxyl alone. He attributed this to the broad-spectrum deleterious effect of mancozeb on natural antagonistic inhabitants of the soil. In our studies, mancozeb at higher concentrations reduced the antibiotic production potential of *B. linens* strain DF-3101 by reducing its growth on the fungicide-amended medium.

The results of this study indicate that metalaxyl is highly efficacious against *P. vignae* in vitro and in vivo and that it is quickly absorbed by roots and translocated acropetally when applied as a soil drench. The possibility of using fungicide mixtures or alternative applications of fungicides seems promising with the effectiveness observed with mancozeb and etridiazole. Biocontrol agents and fungicides at low concentrations could be used as compatible combinations.

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LITERATURE CITED

1. Benson, D. M. 1979. Efficacy and in vitro activity of two systemic acylalanines and ethazole for control of *Phytophthora cinnamomi* root rot of azalea. Phytopathology 69:174-178.
2. Bruck, R. I., Fry, W. E., and Apple, A. E. 1980. Effect of metalaxyl, an acylalanine fungicide, on developmental stages of *Phytophthora infestans*. Phytopathology 70:597-601.
3. Cohen, Y., and Coffey, M. D. 1986. Systemic fungicides and the control of oomycetes. Annu. Rev. Phytopathol. 24:311-338.
4. Cohen, Y., and Reuveni, M. 1983. Occurrence of metalaxyl-resistant isolates of *Phytophthora infestans* in potato fields of Israel. Phytopathology 73:925-927.
5. Davis, R. M. 1982. Control of Phytophthora root and foot rot of citrus with systemic fungicides metalaxyl and phosethyl aluminum. Plant Dis. 66:218-220.
6. Davis, R. M. 1989. Effectiveness of fosetyl-Al against *Phytophthora parasitica* on tomato. Plant Dis. 73:215-217.
7. Ellis, M. A., Grove, G. G., and Ferree, D. C. 1982. Effects of metalaxyl on *Phytophthora cactorum* and collar rot of apple. Phytopathology 72:1431-1434.
8. Elander, L., Merlino, J. A., and McGuire, J. J. 1980. Effect of two new systemic fungicides and ethazol for control of Phytophthora root rot of rhododendron, and spread of *Phytophthora cinnamomi* in propagation benches. Phytopathology 70:1175-1179.
9. Farih, A., Menge, J. A., Tsao, P. H., and Ohr, H. D. 1981. Metalaxyl and efosite aluminum for control of Phytophthora gummosis and root rot on citrus. Plant Dis. 65:654-657.
10. Farih, A., Tsao, P. H., and Menge, J. A. 1981. Fungitoxic activity of efosite aluminum on growth, sporulation, and germination of *Phytophthora parasitica* and *P. citrophthora*. Phytopathology 71:934-936.
11. Farih, A., Tsao, P. H., and Menge, J. A. 1981. In vitro effects of metalaxyl on growth, sporulation, and germination of *Phytophthora parasitica* and *P. citrophthora*. Plant Dis. 65:651-654.
12. Fenn, M. E., and Coffey, M. D. 1984. Studies on the in vitro and in vivo antifungal activity of fosetyl-Al and phosphorous acid. Phytopathology 74:606-611.
13. Fernando, W. G. D., and Linderman, R. G. 1993. Occurrence, distribution, and pathogenicity of the cowpea root and stem rot pathogen, *Phytophthora vignae*, in soils of Sri Lanka. Plant Dis. 77:1158-1164.
14. Fernando, W. G. D., and Linderman, R. G. 1994. Inhibition of *Phytophthora vignae* and stem and root rot of cowpea by soil bacteria. Biol. Agric. Hortic. 12:1-14.
15. Gupta, V. K., and Utkhede, R. S. 1986. Factors affecting the production of antifungal compounds by *Enterobacter aerogenes* and *Bacillus subtilis*, antagonists of *Phytophthora cactorum*. J. Phytopathol. 117:9-16.
16. Han, E. H., Lee, C. H., Sin, C. S., and Lee, E. K. 1982. An investigation on the stem rot of red-bean caused by *Phytophthora vignae* Purss. Res. Rep. Off. Rural Dev. Soil Sci. Fert. Crop Prot. Mycol. Farm Prod. Util. 24:69-71.
17. Hewitt, E. J. 1966. Sand and water culture methods used in the study of plant nutrition. Tech. Commun. 22. 2nd. ed. Commonwealth Agricultural Bureau, London.
18. Hunger, R. M., Hamm, P. B., Horner, C. E., and Hansen, E. M. 1982. Tolerance of *Phytophthora megasperma* isolates to metalaxyl. Plant Dis. 66:645-649.
19. Kao, C. W., and Leu, L. S. 1982. Phytophthora stem rot of cowpea caused by *Phytophthora vignae* Purss in Taiwan. Plant Prot. Bull. 24:189-191.
20. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301-307.
21. Kitazawa, K., Suzui, T., and Yanagita, K. 1979. Pathogenicity of *Phytophthora vignae* Purss to adsuki bean and cowpea [in Hokkaido, Japan]. Ann. Phytopathol. Soc. Jpn. 45:406-408.
22. Nirwan, R. S., and Upadhyaya, J. 1972. Phytophthora blight of cowpea new to India. Indian Phytopathol. 25:162-163.
23. Papavizas, G. C., and Bowers, J. H. 1981. Comparative fungitoxicity of captafol and metalaxyl to *Phytophthora capsici*. Phytopathology 71:123-128.
24. Purss, G. S. 1957. Stem rot: A disease of cowpeas caused by an undescribed species of *Phytophthora*. Queensl. J. Agric. Sci. 14:125-154.
25. Reuveni, M., Eyal, H., and Cohen, Y. 1980. Development of resistance to metalaxyl in *Pseudoperonospora cubensis*. Plant Dis. 64:1108-1109.
26. Sandler, H. A., Timmer, L. W., Graham, J. H., and Zitko, S. E. 1989. Effect of fungicide applications on populations of *Phytophthora parasitica* and on feeder root densities and fruit yields of citrus trees. Plant Dis. 73:902-906.
27. SAS Institute. 1987. SAS/STAT Guide for Personal Computers. Version 6 ed. SAS Institute, Cary, NC.

28. Sivakadadcham, B., and Fernando, W. G. D. 1991. Root and stem rot on greenhouse-grown cowpea caused by *Phytophthora vignae* in Sri Lanka. Plant Dis. 75:215.
29. Smith, P. M. 1979. Chemical control of *Phytophthora cinnamomi* in ornamental woody species. Pages 303-309 in: Proc. Br. Crop Prot. Conf. Pests Dis.
30. Tey, C. C., and Wood, R. K. S. 1983. Effects of various fungicides in vitro on *Phytophthora palmivora* from cocoa. Trans. Br. Mycol. Soc. 80:271-282.
31. Timmer, L. W., and Castle, W. S. 1985. Effectiveness of metalaxyl and fosetyl-Al against *Phytophthora parasitica* on sweet orange. Plant Dis. 69:741-743.
32. Utkhede, R. S. 1983. Inhibition of *Phytophthora cactorum* by bacterial isolates and effects of chemical fungicides on their growth and antagonism. J. Plant Dis. Prot. 90:140-145.
33. Utkhede, R. S. 1987. Chemical and biological control of crown and root rot of apple caused by *Phytophthora cactorum*. Can. J. Plant Pathol. 9:295-300.