Effect of Fertilization and Biopesticides on the Infection of *Catharanthus roseus* by *Phytophthora nicotianae*

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ABSTRACT

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Experiments were carried out in a greenhouse to determine the effect of fertilizer concentration (0, 0.5, 1.0, and 2.0× Hoagland solutions) and various commercial biopesticides on the severity of *Phytophthora nicotianae* infection of Madagascar periwinkle. Application of biopesticides and fertilizer concentration significantly influenced the severity of infection, but there was no significant effect from the interaction of these two factors. Overall, disease severity showed a tendency to increase with the concentration of applied fertilizer. Compared with the control plants, disease was significantly less severe in plants that were treated with the biopesticides, except for plants treated with metabolites of *Myrothecium verrucaria* (DiTera). However, only the products containing potassium phosphonates and potassium phosphates (FNX-100 and FNX-2500) provided a satisfactory level of control when compared with either the control plants or those that received any of the other products tested. Additional experiments were carried out in growth chambers to test the effects of increasing fertilizer concentrations in plants that were inoculated with different *P. nicotianae* inoculum levels. In these trials, there was no consistent indication that disease is most severe in plants that received the highest fertilizer concentration even at the highest inoculum level.

Additional keywords: disease control

Phytophthora nicotianae Breda de Haan (syn = *P. parasitica*) is a serious pathogen of at least 80 plant genera of vegetables, fruit, and ornamentals (18,20). Considerable losses in commercial and residential plantings of Madagascar periwinkle (*Catharanthus roseus* (L.) G. Don, a.k.a. 'Vinca') have been attributed to this pathogen in the southern part of the United States (39). Symptoms of *P. nicotianae* infection of periwinkle include the appearance of water-soaked, grayish-brown lesions on shoot tips and leaf petioles, girdling of the main stem, wilting, and necrosis. Plant death may occur within 1 to 2 weeks after the first appearance of symptoms.

P. nicotianae can survive in the soil as chlamydospores or in plant debris (18) and, therefore, persists from season to season unless the soil is disinfested. However, because of restrictions on the use of chemical soil fumigants, other control measures have been employed to control this disease. Growers have primarily relied on fungicides such as metalaxyl (Ridomil and Subdue) to control Phytophthora diseases; unfortunately, the reliance on fungicides is believed to select for resistance in *Phytophthora* populations (6,32,55). Resistance or insensitivity to metalaxyl has been detected in *P. nicotianae* isolated from ornamental hosts (23), as well as isolates of other economically important *Phytophthora* and *Pythium* spp. (14,52,53,61).

Restrictions on the use of soil fumigants and the emergence of metalaxyl-resistant or metalaxyl-insensitive populations have shifted the focus of Phytophthora disease control to alternative strategies, including the use of biological control agents. Several studies already have investigated the efficacy of various fungal and bacterial biocontrol agents against species of *Phytophthora* causing root rots in azalea (*Rhododendron* spp.), citrus (*Citrus* spp.), and pine (*Pinus* spp.) (19,25,34,44,46,57,59,60) as well as against other soilborne pathogens which infect various ornamental and vegetable species (27,31,35,36,38). Biorationals or reduced-risk chemical pesticides also have been considered for disease control in various crops. These types of pesticides are an attractive option for disease control because they minimize environmental risk by having short residual activity, a high degree of selectivity, or a high level of efficacy in small amounts. Phosphates, phosphonates, phosphites, and mono-ethyl phosphonite (one of the breakdown products of fosetyl-Al) belong to a group of phosphorous acid compounds that reportedly suppressed Phytophthora diseases in various crops, including potato, avocado, almond, cherry, and cocoa trees (13,15,17,45,47,63). Disease suppression by phosphorous acid has been attributed to its ability to inhibit the metabolic process, particularly the process of oxidative phosphorylation in susceptible species of Oomycetes (36). Rouhier et al. (51), presented some evidence that phosphorous acid can induce natural defense mechanisms in plants; according to their study, exposure of *P. capsici* to phosphonates resulted in the production of water-soluble cell wall fractions, which, when applied to tobacco, stimulated the synthesis of capsidiol, a naturally produced antimicrobial compound.

According to Chase and Poole (10), the application of fertilizers or certain nutrients can reduce disease severity in plants either by directly inhibiting the pathogen or by making the host less susceptible to pathogen attack, possibly through the formation of physical barriers to pathogen colonization. Several studies have demonstrated the mitigating effect of plant nutrition on disease development, including Pythium root rot of poinsettia (*Euphorbia pulcherrima* Willd. Ex Klotzsch) and peperomia (*Peperomia* spp.) (10,42), Phytophthora root rot of alfalfa (*Medicago sativa* L.; 28), and Fusarium wilt and root rot in red clover (*Trifolium pratense* L.; 11).

This study was done to (i) evaluate and compare the efficacy of some commercial biologically based pesticides (biopesticides) and biorational products as control agents against *P. nicotianae* of periwinkle under greenhouse culture, (ii) determine whether fertilization level can enhance the

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efficacy of these products against *P. nicotianae* infection, and (iii) determine the effect of increasing levels of fertilization and inoculum on the severity of *P. nicotianae* infection of periwinkle.

MATERIALS AND METHODS

Periwinkle cv. Parasol was used in all greenhouse experiments. Seed (Geo. W. Park Seed Co., Inc., Greenwood, SC) were sown in flats (35 by 67 cm) containing greenhouse potting medium composed of sphagnum peat, processed pine bark, vermiculite, and perlite (Faffard 4P Mix; Faffard, Inc. Anderson, SC). Seedlings were transplanted into plastic pots (8.8 cm in depth; 10 by 10 cm) at approximately 30 days after sowing (one seedling per pot).

An isolate of *P. nicotianae* (Pn-21) originally isolated from periwinkle by R. J. McGovern (University of Florida) was used in the study. Zoospores were produced following the method described by Kuhajek et al. (37). In this method, 15 mycelial plugs (5 mm in diameter) were taken from 4-day-old *P. nicotianae* cultures growing on V8 juice agar and transferred to petri plates (60 by 15 mm) containing 5 ml of sterile mineral salts solution (MSS). The MSS was prepared by adding Ca $(NO_3)_2 \cdot 4H_2O$ (3.08 g), $MgSO_4 \cdot 7H_2O$ (1.49 g) and KNO_3 (0.51 g) to 1 liter of distilled water. The mixture was autoclaved for 15 min at 121°C at 103.42 kPa. After autoclaving, 1 ml of chelated iron solution was added (composed of EDTA [6.52 g], KOH [0.375 g], and $FeSO_4 \cdot 7H_2O$ [1.24 g]) in 50 ml of water that had been filtered through a 0.2-µm membrane filter (Millipore Corp., Billerica, MA). Petri plates with MSS and mycelial plugs were incubated for 24 h under continuous light at 20°C to induce the production of sporangia. After 24 h, the MSS in the plates was replaced with 5 ml of fresh MSS. The plates were further incubated for 48 h, after which the MSS was poured off and the plugs were rinsed three times with sterile distilled water. Plugs then were covered with 5 ml of sterile distilled water and the plates incubated at 4°C for 20 min, after which they were then returned to the 20°C incubator for 3 h to induce the release of zoospores. To determine the zoospore concentration, three 2-ml samples from the zoospore suspensions were transferred to glass vials and agitated on a vortex (Genie 2; Scientific Industries, Inc., Bohemia, NY) for 60 s in order for the zoospores to encyst. The number of encysted zoospores was counted with the aid of a hemacytometer. The zoospore concentration was adjusted to 12,500 zoospores per 10 ml of suspension based on the counts. Just prior to inoculation, a second sample of the inoculum (zoospore suspension) was checked under a stereo microscope for actively swimming zoospores to make sure that the inoculum was viable.

An experiment was performed in the greenhouse to determine the effect of fertilization and test material application on the severity of *P. nicotianae* infection. The study design was a completely randomized two-way factorial with five replicates per treatment. Each experimental unit consisted of a single potted periwinkle plant, as described above. Treatment factors consisted of nine test materials plus an untreated control) and four fertilizer concentrations (0 [no fertilizer], 0.5, 1.0, and 2.0× standard Hoagland solution). Treatments were based on a modified Hoagland nutrient solution (30). The 1.0× treatment contained the following essential plant elements: N at 200 mg liter^{-1} (79% NO_3-N and 21% NH_4-N), P at 62 mg liter^{-1}, K at 168 mg liter^{-1}, Ca at 120 mg liter^{-1}, Mg at 49 mg liter^{-1}, S at 64 mg liter^{-1}, Fe at 1 mg liter^{-1}, Mn at 500 µg liter^{-1}, B at 500 µg liter^{-1}, Zn at 50 µg liter^{-1}, Mo at 50 µg liter^{-1}, and Cu at 20 µg liter^{-1}, derived from KNO_3, KH_2PO_4, $MgSO_4$, $Ca(NO_3)_2$, NH_4NO_3, $NH_4H_2PO_4$, H_3BO_3, H_2MoO_4, FeEDTA, MnEDTA, ZnEDTA, and CuEDTA. A 10.0× stock solution was prepared and dilutions of the concentrated stock were made with elements proportionally diluted to yield solutions of 100 mg liter^{-1} (0.5×), 200 mg liter^{-1} (1.0×), and 400 mg liter^{-1} (2.0×) N. Water treated using a reverse osmosis system was used to prepare the nutrient solutions and it was used for the 0× treatment. Nutrient solution pH after dilution was adjusted to pH 5.8 to 6.0 with NaOH or HCl. The biopesticides used and their application rates and frequency are listed in Table 1. Plants were treated with the various biopesticides according to the label or distributor recommendations (i.e., during [mixed with the growing medium before transplanting] or immediately after transplant, or at regular intervals during the experimental period). Actigard was applied as a foliar spray using the 0- to 2-weeks-after transplant rate for tomato (24 ml/ha) and was applied 1 week after transplant. DieHard was applied to bare roots at transplanting by dipping roots into the gelatinous material. DiTera, MBI600, and Primastop were applied once in 100 ml per plant, 1 week after transplanting and in the case of DiTera, applied weekly throughout the experiment. FNX-100 and FNX-2500 were applied in 100 ml per plant on a biweekly basis. Mycostop was applied once at transplanting at a rate of 0.55 liter per 929 cm^2 of soil surface. SoilGard was incorporated into the potting medium at transplanting. Plants were fertilized with 100 ml of modified Hoagland solution every week for 5 weeks, starting at 7 days after transplant. The experiment was performed twice.

Plants were inoculated with zoospores 10 days after transplant for trial 1 and 12 days afterward for trial 2 of this experiment. All test plants were watered in excess at least 24 h before inoculation. Inoculated plants received 10 ml of zoospore suspension containing approximately 12,500 zoospores. The zoospore suspension was applied onto the soil adjacent to the base of the plant using a pipette. Noninoculated control plants received 10 ml of sterile deionized water applied in a similar manner. To enhance conditions favorable for disease development, inoculated and control plants were kept under wet conditions for three days by adding water to plastic saucers under each pot. Conditions in the greenhouse during the experimental period were approximately 24°C and 96% relative humidity. The average light intensity at the greenhouse at midday was 1,120 µmol s^{-1} m^{-1}.

The severity of *P. nicotianae* infection was assessed 21 days after inoculation using an ordinal 0-to-4 scale that was based on the aboveground symptoms of disease: 0 = no disease, 1 = presence of stem lesion or girdling, 2 = stem lesion or girdling plus wilting of leaves adjacent to the lesion, 3 = stem lesion or girdling plus

Table 1. Materials tested against *Phytophthora nicotianae* on periwinkle

Product name	Active ingredient	Manufacturer	Application rate
Actigard 50 WG	Acibenzolar-*S*-methyl (50% a.i.)	Syngenta Crop Protection	84 mg/liter of water; applied once
DieHard	Endo and ectomycorrhizae fungi	Horticultural Alliance, Inc.	61 g/liter of water; applied once
DiTera WDG	Dried fermented solids and solubles of *Myrothecium verrucaria* strain AARC-0255 (90% a.i.)	Valent Biosciences	2 g/liter of water, applied weekly
FNX-100	Dipotassium phosphate, dipotassium phosphonate (30.2% a.i.)	Foliar Nutrients, Inc.	1% volume:volume; applied biweekly
FNX-2500	Dipotassium phosphate, dipotassium phosphonate; Mn, Zn, Cu (30.2% a.i.)	Foliar Nutrients, Inc.	1% volume:volume; applied biweekly
MBI600	*Bacillus subtilis* MBI 600 (2.75% a.i.)	Microbio Ltd.	0.1% weight:volume; applied once
Mycostop	*Streptomyces griseoviridis* strain K61 (10^8 CFU/g)	AgBio Development, Inc.	0.03 g/liter of water; applied once
Primastop	*Gliocladium catenulatum* strain J1446 (37% a.i.)	AgBio Development, Inc.	0.5% solution; applied once
SoilGard 12G	*Trichoderma* (*Gliocladium*) *virens* GL-21 (12% a.i.)	Certis USA LLC	21 g per 0.03 m^3 of potting medium

wilting of the lower and upper leaves, and 4 = death of the plant. Nonparametric data analysis was done with SAS Proc Mixed (SAS Institute, Cary, NC), as detailed previously (54). Relative treatment effects and their confidence interval limits were calculated by the SAS LD_CI macro (7). Separate analyses were done for each repetition of the experiment.

A growth-chamber experiment was done to investigate the effects of fertilization at different inoculum levels on the severity of *P. nicotianae* infection on periwinkle. Specifically, the goal was to determine the optimum fertilizer level for suppressing disease in plants that received various levels of *P. nicotianae* inoculum. Experimental units consisted of single potted periwinkle plants as described above. Growth-chamber conditions were 23 and 30°C night and day temperatures, respectively, with an average relative humidity of 85%. The growth chamber was programmed to provide a light intensity of 420 μmol s^{-1} m^{-1} at midday. The plants were fertilized with 0, 0.5, 1.0, and 2.0× modified Hoagland solutions every week for 3 weeks prior to inoculation, and a week after inoculation with 0 (control), 5,000, 10,000, or 20,000 *P. nicotianae* zoospores per plant. Zoospore suspensions were prepared as described above. The inoculum levels used were approximately half (5,000), nearly the same (10,000), or approximately double (20,000) the inoculum concentration (12,500) that was used in the greenhouse experiment.

Fertilizer and zoospore treatment levels were arranged as a completely randomized two-way factorial (four fertilization levels plus four zoospore concentrations per plant) replicated five times, and the entire experiment was done twice. Disease severity was assessed using the 0-to-4 rating scale at 7, 14, and 21 days after inoculation. Each trial was analyzed separately as a two-way factorial repeated-measures design using nonparametric methods. Treatments not inoculated with zoospores were deleted from the data set before analysis. Test statistics were calculated in SAS with the F2_LD_F1 macro, and relative treatment effects estimated using the LD_CI macro (7). Contrast statements within Proc Mixed were used to test linear trends in relative treatment effects over time.

Table 2. Median disease severity ratings for periwinkle in response to tested products[a]

	Standard Hoagland solution concentration			
Product	**0×**	**0.5×**	**1.0×**	**2.0×**
Actigard	0	4	3	1
DieHard	1	2	3	4
DiTera WDG	0	4	4	4
FNX-100	0	0	0	0
FNX-2500	0	0	0	0
MBI600 Subtilex	0	0	0	4
Mycostop	3	0	4	4
Primastop	0	0	4	4
SoilGard 12G	0	4	2	2
Untreated	4	4	4	4

[a] Median ratings for both trials were similar hence, only the ratings from trial 1 are shown. Disease severity was rated on a 0-to-4 ordinal scale. Plants received 100 ml of standard Hoagland solution every week for 5 weeks, beginning 7 days after transplant. Plants were inoculated with 10 ml of a suspension of *Phytophthora nicotianae* zoospores (about 12,500 zoospores/ml) 10 to 12 days after transplant.

RESULTS

Median disease severity varied with the biopesticides applied and fertilization level in the greenhouse experiment (Table 2); there were statistically significant differences among the main treatment levels ($P < 0.01$) but no significant biopesticide–fertilization level interaction effect ($P > 0.20$). Disease severity was significantly lower in plants that were treated with the biopesticides compared with the control plants ($P < 0.05$), except for those that had been treated with DiTera ($P > 0.16$). However, FNX-100 and FNX-2500 were the only two biopesticides which stood out in terms of disease control when compared with either the untreated plants or those that received any of the other biopesticides tested (Figs. 1 and 2). Overall, disease severity showed a tendency to increase with the concentration of applied fertilizer (Fig. 1), although the difference in disease severity between untreated plants and those fertilized with 0.5× Hoagland solution was not statistically significant ($P > 0.25$).

In the growth-chamber experiment, disease severity was noticeably lower in trial 1 compared with trial 2 (Table 3), which may account in part for the conflicting test statistics between experiments for the effects of fertilizer concentration and inoculum level on disease severity (Table 4). In both trials, however, there was no significant interaction effect between fertilizer concentration and inoculum level. There was a significant increase in disease severity over time in both trials (Table 4). Plots of relative treatment effects (Fig. 3) illustrate the trends over time and the relationships between the different fertilizer concentrations and inoculum levels.

In trial 1 of the growth-chamber experiment, the inoculum level had no effect

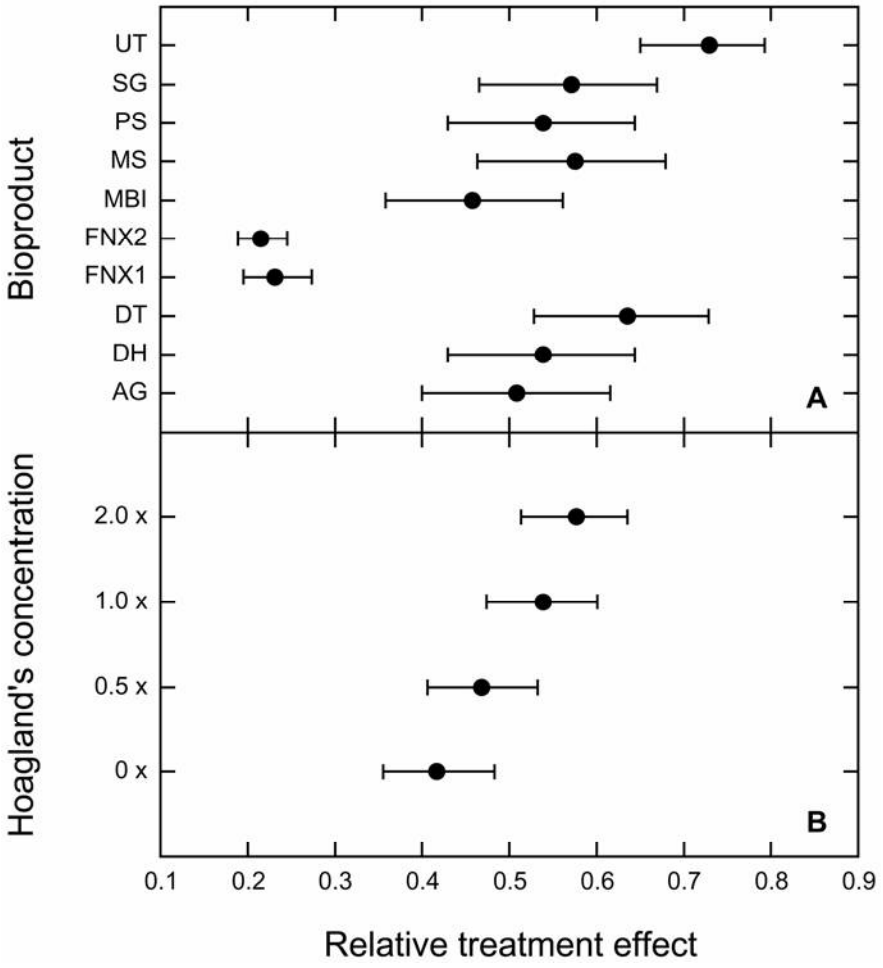

Fig. 1. Treatment effects for **A,** biopesticides and **B,** different concentrations of Hoagland solution on *Phytophthora nicotianae* infection of periwinkle for the first of two independent trials. Results for the second trial were similar statistically. AG = Actigard, DH = DieHard, DT = DiTera, FNX1 = FNX-100, FNX2 = FNX-2500, MBI = MBI600, MS = Mycostop, PS = Primastop, SG = SoilGard, and UT = untreated control.

on disease severity (Table 4; Fig. 2). Disease severity increased over the 3 weeks postinoculation; by 21 days postinoculation, there were no differences in disease severity among plants inoculated with 5,000, 10,000, or 20,000 zoospores ($P > 0.25$). On the other hand, in trial 2 (in which disease severity was overall higher), disease severity at 21 days postinoculation was significantly higher in plants inoculated with either 10,000 or 20,000 zoospores ($P < 0.0001$) than in those inoculated with 5,000 zoospores (Fig. 2). Also, the linear trend in disease severity after inoculation with 5,000 zoospores per plant was significantly different ($P < 0.001$) from the trends seen after inoculation with 10,000 and 20,000 zoospores per plant (Fig. 2). The linear trend in disease severity did not differ between plants inoculated with 10,000 or 20,000 zoospores ($P = 0.8077$).

In trial 1 of the growth-chamber experiment, the fertilization effect was significant (Table 4). By 21 days postinoculation, disease severity after treatment with 2.0× modified Hoagland solution was significantly higher ($P < 0.01$) than in plants treated with the other fertilizer concentrations (Fig. 2). Disease severity level at 21 days postinoculation was not different among plants that received 0, 0.5 or 1.0× Hoagland solution ($P > 0.13$). The linear trend in disease severity over time was different only between nonfertilized plants and those treated with 2.0× Hoagland solution ($P = 0.0002$). In trial 2, the only significant differences in disease severity 21 days postinoculation were between the nonfertilized plants and those treated with 1.0× Hoagland solution ($P = 0.0079$). There were no differences among fertilization treatment in linear trend ($P > 0.05$).

DISCUSSION

Biweekly applications of phosphonate-containing products resulted in significant disease suppression in periwinkle plants inoculated with *P. nicotianae*. These results agree with the findings of other studies that the application of phosphonates can suppress Phytophthora diseases, such as late blight of potato (caused by *P. infestans*), avocado stem canker (caused by *P. citricola*), avocado root rot (caused by *P. cinamomi*), canker in almond and cherry trees (caused by *P. cambivora*), and black pod disease of cocoa (caused by *P. palmivora* and *P. megakarya*) (13,15,17,45, 47,58,63). Control of Phytophthora blight in periwinkle with phosphite also has been reported by Banko and Hong (3); according to their study, foliar application of phosphites controlled blight in periwinkle plants that previously have been sprayed with zoospores of *P. nicotianae* whereas drench applications of phosphites were ineffective in controlling the foliar blight. In our study, drench applications of phosphonates controlled infection in plants that were growing in soil that had been infested with *P. nicotianae* zoospores. From these studies, we conclude that *Phytophthora* infection can be controlled with the tested phosphate or phosphonate-based products. The materials tested may provide disease control through direct inhibition of the pathogen (12) or inhibition of fungal growth (21,29,56), as has been proven with similar materials in other pathosystems.

The application of the other test materials (Actigard, Diehard, DiTera, MBI600, Mycostop, Primastop, and Soilgard) did not result in suppression or reduction of *Phytophthora* infection of periwinkle in this study. Lack of control from the application of Mycostop or SoilGard also has been reported for other host–pathogen combinations, including *Pythium ultimum* on vinca (8), *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (50) and *P. aphanidermatum* on cucumber (48), and *P. tracheiphilum* on Chinese cabbage (41).

However, there are studies that reported satisfactory level of control with Actigard, DiTera, Primastop (Prestop), and *Bacillus subtilis* (the active ingredient of MBI600) against other pathogens that attack other crop species; Actigard has been shown to control bacterial canker of tomato (4), Xanthomonas blight of onion (26), fire blight of apple (5), and infections by *Didymella bryoniae* and *Sclerotinia sclerotiorum* on melon (9); DiTera reportedly suppressed populations of root-knot, cyst, sting and burrowing nematodes (22,62); Primastop (Prestop) controlled *F. oxysporum* f. sp. *radicis-cucumerinum* (50) and *P. aphanidermatum* in greenhouse cucumbers (48); and *B. subtilis* reduced the severity of bean rust in field tests (2).

Potential reasons why these biopesticides reduced disease severity in other pathosystems but failed to control *P. nicotianae* in this particular study are numerous and may vary among products; these may include high disease pressure, introduction of the pathogen before the biological control agent has effectively colonized the plant root system, low dosage or low application rates, suboptimal environmental conditions for the growth and colonization of the biological agent, or narrow specificity (27,35,49). Among the biopesticides tested, Primastop and Mycostop both are labeled for the control of Phytophthora root rot in vinca, SoilGard and FNX-100 are labeled for Phytophthora root rot of ornamentals but not specifically for vinca, and Actigard, DieHard, MBI600, and DiTera are not labeled for Phytophthora diseases. FNX-2500 is an experimental material that does not yet have a label, but does provide control of *Phytophthora* spp. as seen here. Biological

Table 3. Median disease severity rating of periwinkle in response to inoculation with different numbers of *Phytophthora nicotianae* zoospores and fertilization with different concentrations of standard Hoagland solution

	Disease rating (dpi)[a]		
Zoo./conc.[b]	**7**	**14**	**21**
Trial 1			
5,000			
0	0	0	0
0.5x	0	0	0
1.0x	0	0	0
2.0x	0	0	0
10,000			
0	0	0	0
0.5x	0	0	0
1.0x	0	0	0
2.0x	0	3	4
20,000			
0	0	0	0
0.5x	0	0	0
1.0x	0	0	2
2.0x	0	0	3
Trial 2			
5,000			
0	0	0	0
0.5x	0	0	0
1.0x	0	0	2.5
2.0x	0	0	0
10,000			
0	1	1	2
0.5x	1	2	4
1.0x	1	2	4
2.0x	1	2	4
20,000			
0	1	2.5	4
0.5x	1	2.5	4
1.0x	1	4	4
2.0x	1	3.5	4

[a] Disease severity was rated on a 0-to 4-ordinal scale at 7, 14, and 21 days postinoculation (dpi).
[b] Zoospore concentration (Zoo.) = number of zoospores per milliliter, followed by Hoagland solution concentration (conc.).

Fig. 2. Severity of *Phytophthora nicotianae* infection of periwinkle **A,** without and **B,** with FNX-100 treatment at all fertilization levels.

control agents or biorationals can be effective in one pathosystem and yet ineffective in another (27); hence it is important to determine the particular system, including cultural system as well as crop, in which these products should be used and at what rate they should be applied in order for them to effect control.

Table 4. Test statistics for the effects of zoospore and fertilizer levels on the severity of *Phytophthora nicotianae* infection of periwinkle

Effect	Analysis of variance-type statistic[a]			
	df_N	df_D	*F*	*P* value
Trial 1				
A (Inoculum level)[b]	1.9089	20.653	0.88324	0.42400
B (Fertilizer concentration)[c]	2.4682	20.653	7.5406	0.00217
T (Days after inoculation)[d]	1.4518	∞	10.255	0.00026
A × B	3.8338	20.653	1.9104	0.14874
A × T	2.4115	∞	1.0358	0.36500
B × T	3.1257	∞	2.9051	0.03137
A × B × T	4.6483	∞	1.9524	0.08782
Trial 2				
A	1.9915	36.999	12.921	0.00006
B	2.8658	36.999	1.6928	0.18710
T	1.5440	∞	84.653	<0.00001
A × B	5.3383	36.999	0.23471	0.95160
A × T	2.9558	∞	10.158	<0.00001
B × T	4.4371	∞	0.86864	0.49082
A × B × T	7.4093	∞	0.59693	0.76849

[a] Abbreviations: df_N = numerator degrees of freedom and df_D = denominator degrees of freedom.
[b] Plants were fertilized with 0, 0.5, 1.0, or 2.0× standard Hoagland solution once per week for 3 weeks before inoculation with *P. nicotianae* zoospores, and a week after inoculation.
[c] Each pot (one plant per pot) was inoculated with 5,000, 10,000, or 20,000 *P. nicotianae* zoospores.
[d] Disease severity was assessed at 7, 14, and 21 days after inoculation.

Although fertilizer concentration did not have an effect on the efficacy of any of the biopesticides used in this study, we observed the tendency of disease to be more severe in plants that received 1× or 2× concentration of fertilizer compared with 0× and 0.5× for most biopesticides tested (except for the phosphonates). However, experiments aimed at determining the effect of increasing fertilizer concentrations on the severity of *Phytophthora* infection in plants failed to show the clear-cut relationship between fertilization level and disease severity at the three inoculum levels tested. Other studies on the effect of fertilizer level on Phytophthora root rot have not consistently shown that higher fertilizer levels caused plants to have more severe infections or greater incidence of disease. In soybean, the incidence of Phytophthora root rot reportedly increased with increasing application rates of 8-32-16 NPK fertilizer (16). However, an experiment by Alva et al. (1) indicated that N or P fertilization had no significant effect on the severity of Phytophthora root rot of alfalfa (*M. sativa* L.). Studies on the effect of fertilizer levels on the severity of Pythium root rot also reported opposite results. In a study by Moorman (42) involv-

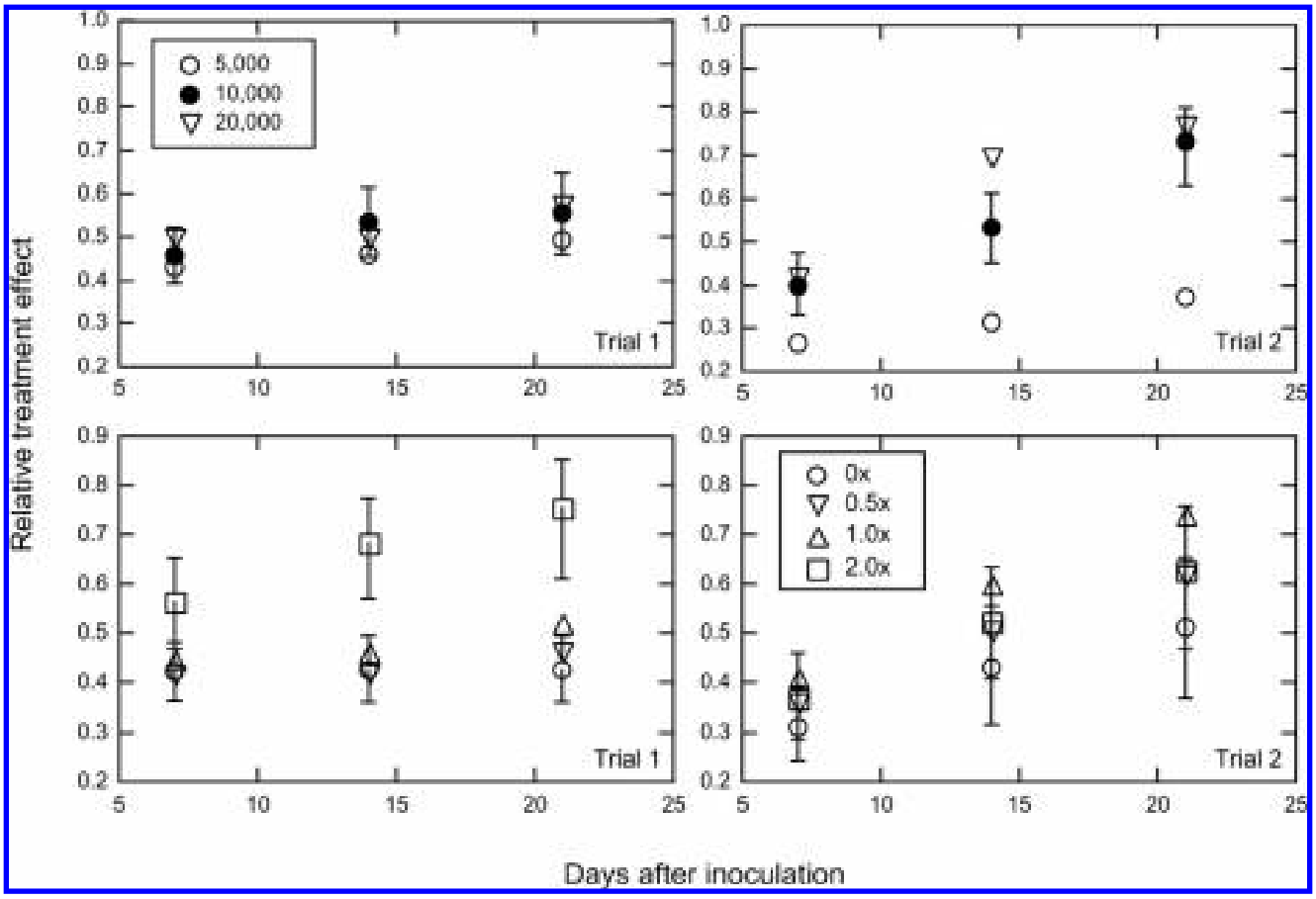

Fig. 3. Relative treatment effects (r.e.) for fertilization and zoospore levels on *Phytophthora nicotianae* infection of periwinkle. Plants were fertilized with 0, 0.5, 1.0, or 2.0× Hoagland solution once per week for 3 weeks prior to inoculation with *P. nicotianae* zoospores (5,000, 10,000, or 20,000 zoospores per plant), and again a week after inoculation. Disease severity ratings were done 7, 14, and 21 days after zoospore inoculation. Data are shown for two trials. The upper panels show the r.e. in response to zoospore number; the lower panels represent the r.e. in response to fertilization level. Confidence interval widths (95%) for r.e. are shown for the 10,000 zoospore per plant and 0× and 2.0× Hoagland solution treatments. Note that relative treatment effects are calculated independently for each trial, and their comparison between trials is not legitimate.

ing N-P-K fertilizer solutions that contained increasing amounts of nitrogen, mortality caused by Pythium root rot was higher in poinsettias that received fertilizers with the higher nitrogen content. However, in a study by Chase and Poole (10) where they compared the effect of varying concentrations of 19-3-10 NPK fertilizer, they observed that Pythium root rot was more severe in plants that received the lowest fertilizer concentration. Based on the results of our study and of other studies mentioned here, it would be difficult to make a recommendation for fertilization that would minimize the impact of diseases caused by these pathogens.

Although there are reports of the effect of increasing *Phytophthora* spp. inoculum levels on the incidence and severity of disease in both greenhouse (40) and field experiments (43), our experiment did not indicate a correlation between inoculum level and disease severity. Disease levels were not significantly different in plants that were inoculated with different inoculum levels, possibly because the inoculum levels chosen were too close to each other, the inoculated area was small (increased likelihood of zoospore and root contact), and the cultural conditions (growth chamber temperature, relative humidity, and soil moisture) provided were optimal for initial infection, secondary spore production, and secondary infection in the same plant. Mitchell and Kannwischer-Mitchell (40) offered the following mechanisms by which severe disease can occur: (i) a slow-progressing infection caused by a small number of zoospores suddenly progresses rapidly (due to some external conditions), (ii) low disease level caused by a small number of zoospores is followed by secondary spore production and significant secondary infection and disease in the same plant, or (iii) rapid and severe infections by high amounts of inoculum occur at many susceptible sites on the crown or root area. It is possible that, at lower inoculum concentrations, a threshold may be identified at which particular biopesticides may be more effective; however, at the inoculum levels tested, this did not occur.

These trials were conducted in the greenhouse in order to determine what types of materials might be useful to growers in field and greenhouse production systems. Many growers currently depend on metalaxyl-based fungicides for control of Pythium and Phytophthora diseases in ornamental and vegetable production and achieve acceptable levels of control when the inoculum present in the field is not resistant and the weather conditions do not prevent or overcome effective fungicide applications. Due to the increasing occurrence of resistant populations (24,33), it is necessary to further evaluate materials that have the potential to partner with existing control practices and materials.

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