Efficacy of *Penicillium funiculosum* as a Biological Control Agent Against Phytophthora Root Rots of Azalea and Citrus

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ABSTRACT

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Penicillium funiculosum was evaluated in the greenhouse for its ability to suppress Phytophthora root rots of azalea (*Rhododendron* spp.) and sweet orange (*Citrus sinensis*) as measured by increased shoot and root growth. The antagonist was grown in a wheat bran-peat moss (1:1) medium, which was then mixed into a peat-perlite (3:1) planting mix at a concentration of 0.7% (wt/vol). Rooted azalea cuttings or sweet orange seedlings were transplanted into the planting mix, which was inoculated with *Phytophthora* 5 to 7 days later. In most of the tests, *P. funiculosum* effectively increased the shoot growth and root weight of azalea in media infested with *P. parasitica* and those of sweet orange in media infested with *P. citrophthora*. It also reduced, to some degree, root rots caused by *P. cinnamomi* on azalea and by *P. parasitica* on sweet orange. Three subisolates of *P. funiculosum* (T327H, T327L, and T327S) exhibited different degrees of root rot suppression; T327S provided the best control. The population density of each of these subisolates increased in the planting mix during the first 4 weeks and decreased thereafter. Mixing the *P. funiculosum* bran-peat inoculum into the planting mix was a more effective method for delivering the biocontrol agent than dipping the plant root systems into a *P. funiculosum* spore suspension before transplantation. *P. funiculosum* was also effective in increasing plant growth when the bran-peat inoculum was used at concentrations lower than 0.7%. *P. funiculosum* inoculum applied at a concentration of 0.35% twice during a 12-week period was more effective in the biocontrol of azalea root rot caused by *P. cinnamomi* than when it was applied only once. *P. funiculosum* also reduced sweet orange root rot caused by *P. citrophthora* in some other planting mixes.

Additional keywords: soilless mix.

Phytophthora root rots and crown rots of fruit trees and woody ornamentals are common and destructive diseases in both nursery and field plantings. The disease often originates on seedlings, rooted cuttings, or other forms of juvenile plants in the planting mix in containers and later causes severe damage in the landscape or orchards (47). Azaleas and citrus are two such groups of woody perennials severely damaged by root rots caused by *Phytophthora* spp. Since its first report in 1929, Phytophthora root rot of azaleas and the related rhododendrons has been found in nurseries throughout the United States (7,28). Several *Phytophthora* species cause root rot; *P. cinnamomi* Rands is the most important species in the root rot syndrome of rhododendrons, and *P. parasitica* Dastur (syn. *P. nicotianae* Breda de Haan) is a common root rot pathogen of greenhouse azaleas (26). *P. parasitica* and *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian are major fungal pathogens of citrus roots, and the disease is one of the most serious problems in citrus nurseries and orchards worldwide (45).

To date, Phytophthora root rots have been partially controlled by cultural means (8,45). Resistant cultivars are available (7,22, 23,28); however, predisposing factors such as flooding may reduce or negate resistance (9). Also, cultivars of azalea are chosen for commercial use on the basis of characteristics such as flowering habit, color, and ease in forcing rather than on any consideration of disease resistance (6,30). Chemical control provided by highly effective Oomycetes-specific fungicides, such as metalaxyl and fosetyl Al, has been successful (5,15,19,27) but is not always desirable because of the high cost of application, potential hazards to the environment, toxicity to crops, and the development of fungicide-resistant strains (13).

Although biological control of root-disease fungi has been studied by many researchers, few studies have been made on the control of *Phytophthora* by the use of specific antagonistic hyphomycetous fungi (40). Some recent studies have demonstrated the reduction in Phytophthora root rots by species of *Aspergillus* (44), *Gliocladium* (42), *Myrothecium* (20), *Penicillium* (35,44), and *Trichoderma* (29,42).

Penicillium funiculosum Thom is a widely distributed and common soil-inhabiting hyphomycete (37). The activity of *P. funiculosum* against several species of *Phytophthora* has been reported (49). It has been shown to control Phytophthora gummosis (bark infection) on stems of sweet orange seedlings and lemon trees by reducing the size of lesions caused by *P. citrophthora* and *P. parasitica* (43). In preliminary experiments, the severity of azalea root rot caused by *P. cinnamomi* or *P. parasitica* was reduced when *P. funiculosum* grown in a bran-peat medium was incorporated into peat-perlite planting mix prior to *Phytophthora* inoculation (49).

The potential for biocontrol of root rots of woody ornamentals and fruit trees in the nursery is aided by the use of soilless planting mixes in containers. While it is difficult to establish foreign biocontrol agents in a stable ecosystem such as soil (11,14), it is easier to establish them in a soilless mix that has a number of vacant ecological niches (11). Planting mixes, although not sterile, are generally less complex microbiologically than soil. These soilless mixes can be inoculated with antagonists, which then have a reasonable chance of successful establishment. Under

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nursery conditions or in covered and environmentally controlled structures, environmental conditions can be modified to suit the antagonists rather than the pathogens (3,11). Furthermore, with woody ornamentals or fruit trees of high value, the use of relatively expensive or complex control measures may be cost effective and therefore economically feasible (11).

In this study, we examined the potential of *P. funiculosum* for use in the biological control of Phytophthora root rots of azalea and citrus in planting mixes under greenhouse conditions. Portions of this research have been reported previously (17).

MATERIALS AND METHODS

Fungal isolates. The *P. funiculosum* used was originally isolated from the soil of a citrus orchard in Riverside, California, in 1961. Three subisolates of *P. funiculosum* (T327H, T327L, and T327S) were used in some experiments to compare their biocontrol efficiencies. T327S was the original culture, which had been maintained at 8°C. T327H was a natural mutant of T327S, and T327L was from a sector of T327H. Identification to species was based on the monographs by Raper and Thom (38) and Pitt (37). T327S had some morphological features resembling those of *P. pinophilum* Hedgc. (37). Unless otherwise specified, subisolate T327S was used in most of the tests. *P. cinnamomi* isolate B101 (= T602, ex azalea) and *P. parasitica* isolate T593 (ex azalea) were used in the azalea experiments. *P. citrophthora* isolate P1156 (= T590, ex citrus) and *P. parasitica* isolate T131 (ex citrus) were used in the sweet orange experiments.

Preparation of inocula. *P. funiculosum* was grown on a modified medium of Sivan et al (41) consisting of wheat bran, peat moss, and water (2:2:1 by volume). The peat moss used was the light-color type Sunshine (Sun Gro, Bellevue, WA) and was sieved through a 2-mm screen before use. Three hundred cubic centimeters of the medium in 500-ml Erlenmeyer flasks were autoclaved for 1 h on each of two successive days. Five 4-mm-diameter agar disks from the advancing margin of a colony growing on cleared V8 juice agar (V8C) (20% V8 juice, 0.2% $CaCO_3$, clarified by centrifugation, and 1.5% agar) were transferred to each flask. Flasks were incubated at 25°C (±1°C) for 16 to 21 days; the flasks were shaken vigorously every 2 to 3 days to distribute the inoculum and prevent clumping of the medium. Inoculum of *P. funiculosum* consisted of hyphae and conidia produced in the bran-peat mixture.

Medium for producing *Phytophthora* inocula was prepared by adding 10 ml of deionized water to 10 g of hulled millet seeds (*Panicum miliaceum* L.) in 100-mm-diameter glass petri plates, which were then autoclaved for 30 min. Five disks from the advancing margin of a colony of *Phytophthora* growing on V8C agar were transferred to each plate, and the plates were incubated at 25°C for 2 weeks before use.

Test plants. Rooted cuttings of azalea cultivar Chimes (an Indian hybrid type of *Rhododendron* spp.) were provided by Monrovia Nursery (Azusa, CA). The rooted cuttings used in most tests were 2 to 8 months old and had been grown in flats in a peat-perlite (7:3, vol/vol) planting mix under natural light at 24°C (±5°C). Some rooted cuttings were transferred from the flats to 5-cm-square plastic pots before use. Seeds of sweet orange (*Citrus sinensis* (L.) Osbeck 'Madam Vinus') were planted in flats in a peat-sand (1:1, vol/vol) UC planting mix (1). Seedlings used in most tests were 2 to 3 months old. However, all rooted cuttings or seedlings used in any given experiment were uniform in age and size.

Planting mix. A 3:1 (vol/vol) mixture of nonsterile Canadian sphagnum peat moss (Sunshine, light type, H = 2) and perlite sponge rock (Therm-O-Rock, Chandler, AZ) was used in most tests. The pH of the mix was 4.3 to 4.5. In addition to the peat-perlite mix, three other planting mixes, peat-vermiculite (1:1), peat-sand (1:1), and pine bark-sand (1:1) (or pine bark-peat-sand-perlite at 3:2:1:1), were used in some tests involving sweet orange.

Incorporation of *P. funiculosum* into the planting mix. The *P. funiculosum* bran-peat inoculum was mixed thoroughly with peat-perlite planting mix at 0.7% (wt/vol) by the "pyramid" method of Farih et al (19). Briefly, the inoculum was spread evenly over a layer of the planting mix and thoroughly mixed by hand. This was followed by making pyramids or mounds back and forth three times. The control treatment consisted of the planting mix plus 0.7% noninoculated bran-peat mixture. In some experiments, planting mix alone was used as an additional control. One rooted azalea cutting or one sweet orange seedling was planted in each 10-cm-square pot (approximately 800 cm^3) containing 600 to 700 cm^3 of planting mix. A 15-cm-diameter, 2.5-cm-high saucer was placed under each pot, and the plants were watered immediately.

Inoculation of plant roots with *P. funiculosum* by root dip. An alternative method was also tested as a delivery system for *P. funiculosum* inoculum. Conidia of *P. funiculosum* were collected in distilled water from 7-day-old cultures grown on V8C agar medium in 90-mm-diameter petri plates. The conidial suspension was adjusted to 4×10^6 to 5×10^7 per ml. The root system of each test plant was dipped into the conidial suspension for 2 min before it was transplanted into the planting mix.

Inoculation with *Phytophthora*, watering method, and data recording. *Phytophthora*-colonized millet seeds (1 or 2 g) were added to each pot in a layer about 1 cm below the surface 5 to 7 days after the test plants were transplanted into *P. funiculosum*-infested planting mix. The plants received water containing Hoaglund's solution generally twice a week, once lightly from the top during the week and once before the weekend by heavy watering. Saturation of the planting mix was maintained for about 2 days by filling the pot and saucer below with water (48). The matric potential of the planting mix was maintained between 0 and −10 kPa throughout the experiment. At the beginning of the experiment, the shoot apex or apices of each test plant were marked with indelible india ink, and the increase in new shoot growth was measured and recorded periodically and at the end of the experiment. Test plants were harvested at 6 or 8 weeks; planting mix was washed off roots; tops and roots were separated and oven dried at 85°C for 24 h; and individual dry weights were recorded. For azalea plants, degrees of root rot were rated on a scale of 1 to 7 (1 = 0% discolored, and 7 = 100% discolored). For sweet orange seedlings, the numbers of white root tips (48) were counted after plants were harvested.

Quantitation of *P. funiculosum*. *P. funiculosum* population densities were quantified by a dilution plating method with RB-M2 medium, a modification (46) of Martin's rose bengal-streptomycin medium (34). Samples (5 g per pot) were collected from each of five pots per treatment. The five samples were pooled and then divided into two subgroups. A 10-g sample was taken from each subgroup and suspended in 100 ml of sterile distilled water in a glass bottle. Serial dilutions were prepared immediately to give final dilutions of 10^{-5} to 10^{-8}, depending on the experiments and treatments, and 1-ml aliquots of the final dilutions were added to 90-mm-diameter petri plates (three plates per replicate). Approximately 15 to 16 ml of RB-M2 medium maintained at 45°C was poured into each plate, and the plates were gently swirled to distribute the suspension uniformly. Also, part (2 g) of each peat-perlite sample was oven dried at 105°C, and the percent moisture was determined. The *P. funiculosum* populations were expressed as propagules (CFU) per gram (ppg) of oven-dried peat-perlite mix.

Experimental designs and statistical analyses. Most greenhouse experiments were conducted at least twice. Five single-plant replicates of each treatment were used in each experiment. Pots were arranged on the greenhouse bench in a randomized complete block design. Most experiments were conducted with

factorial combinations of treatments. Analyses of variance and single degree of freedom contrasts were calculated with MSTAT 4.0C (Michigan State University, East Lansing), and mean separations were done based on the *F* tests from the analysis of variance or Fisher's least significant difference test.

RESULTS

Reduction of Phytophthora root rot of azalea. Reduction in root rot and increase in shoot growth and root weight of azalea, in peat-perlite planting mix containing 0.7% bran-peat inoculum of *P. funiculosum*, were evaluated by using two *Phytophthora* spp., *P. cinnamomi* and *P. parasitica*. The analysis of variance for the first experiment revealed that the main effect of *P. funiculosum* was significant for both new shoot growth ($P = 0.001$) and root weight ($P = 0.028$) of azalea. Neither the main effect of *Phytophthora* nor the interaction of *P. funiculosum* with *Phytophthora* was significant. On the basis of single degree of freedom contrasts, *P. funiculosum* did not significantly reduce root rot caused by *P. cinnamomi;* however, new shoot growth of the *P. funiculosum* + *Phytophthora* treatment was about the same as that of the noninoculated control (Table 1). Azalea root rot caused by *P. parasitica* was significantly reduced by *P. funiculosum*. The plants in the *P. funiculosum* + *Phytophthora* treatment had less root rot and significantly greater new shoot growth and root weight than those of the treatment with *Phytophthora* alone (Table 1). In the second experiment, root rot caused by *P. cinnamomi* was very severe, and *P. funiculosum* was less effective in disease reduction than in the previous experiment. However, *P. funiculosum* again significantly reduced the disease caused by *P. parasitica* in the repeat experiment. The new shoot growth measurements of azalea cuttings grown in the *P. funiculosum* + *Phytophthora* treatment and the treatment with *Phytophthora* alone were 57.6 and 5.2 mm, respectively. In both experiments, azaleas in the treatment with *P. funiculosum* alone grew considerably better than those in the noninoculated control treatment.

Seven additional greenhouse experiments on azalea root rot were conducted to evaluate the efficacy of *P. funiculosum*. *P. cinnamomi* was used in all seven experiments, but *P. parasitica* was included in only five of them. *P. funiculosum* reduced root rot caused by *P. cinnamomi* to some degree in six of the seven experiments and that caused by *P. parasitica* in four of five of these subsequent experiments. Again, as in the two initial experiments, azalea cuttings that received *P. funiculosum* alone had greater new shoot growth and root weight than those of the noninoculated control, although the differences were usually not significant. Two noninoculated control treatments, one with and one without the noninoculated bran-peat in the planting mix, were incorporated in three of the experiments to determine the effect of bran-peat on the growth of test plants. There were no significant differences in new shoot growth and top and root weights between these two controls.

Reduction of Phytophthora root rot of sweet orange. The ability of *P. funiculosum* to reduce sweet orange root rot caused by *P. citrophthora* or *P. parasitica* was evaluated in two greenhouse experiments. In the first, the analysis of variance revealed that the main effect of *P. funiculosum* was significant for new shoot growth ($P = 0.005$) but not for the number of white root tips. Neither the main effect of *Phytophthora* nor the interaction of *P. funiculosum* with *Phytophthora* was significant for either parameter. In the presence of *P. citrophthora*, *P. funiculosum* significantly increased the new shoot growth of sweet orange seedlings, but the increase in number of white root tips was only marginally significant ($P = 0.06$) (Table 2). The improved growth caused by *P. funiculosum* in the presence of *P. parasitica* was slight but was not statistically significant ($P = 0.07$). In another experiment, *P. funiculosum* again greatly reduced root rot caused by *P. citrophthora*. New shoot growth for the *P. funiculosum* + *Phytophthora* and *Phytophthora* alone treatments were 86 and 22 mm, respectively. *P. parasitica* caused only slight disease in this experiment, and reduction of root rot by *P. funiculosum* was also slight and not significant.

Comparison of three subisolates of *P. funiculosum*. The three subisolates of *P. funiculosum* were examined on various agar media including Czapek-yeast extract agar, malt extract agar, and 25% glycerol-nitrate agar, all recipes taken from Pitt (37). They were compared for growth rate, colony morphology, and sporulating ability. The three subisolates were similar in their growth rates. However, they differed in colony color and sporulating ability. T327S produced a green colony with heavy sporulation, T327L produced a whitish colony with light sporulation, and T327H produced a yellow green colony with a sporulation intensity between those of T327S and T327L.

The three subisolates were evaluated in greenhouse tests for their relative abilities to reduce Phytophthora root rots of azalea and sweet orange. All three were used at a concentration of 0.7%. For the experiment on azalea root rot caused by *P. parasitica*, data of which are not reported in detail here, the three subisolates reduced disease to varying degrees. New shoot growth of azalea cuttings in the *P. funiculosum* + *Phytophthora* treatments were 127, 98, and 152 mm for T327H, T327L, and T327S, respectively, compared with 39 mm for the *Phytophthora* alone treatment. The differences were significant ($P = 0.05$) for both T327H and T327S but not for T327L. In the case of *P. cinnamomi,* new shoot growth of azalea cuttings in the *P. funiculosum* + *Phytophthora* treatments were 22, 34, and 35 mm for T327H, T327L, and T327S, respectively, compared with 10.8 mm for the *Phytophthora* alone treatment. However, these differences were not statistically significant.

With sweet orange root rot caused by *P. citrophthora* and *P. parasitica*, the three subisolates also performed differently;

TABLE 1. New shoot growth and root weight of azaleas in the presence of *Penicillium funiculosum* (Pf) and two *Phytophthora* spp. at 8 weeks[x]

	New shoot growth (mm)[y]			Root weight (mg)		
Phytophthora sp.	No Pf	With Pf	Mean	No Pf	With Pf	Mean
None	28.6	70.0*	49.3	70.2	81.6	75.9
P. cinnamomi	8.0	28.4	18.2	36.8	57.9	47.3
P. parasitica	9.8	69.0*	39.4	38.4	99.2*	68.8
Mean[z]	15.5 b	55.8 a		48.5 b	79.6 a	

[x] *P. funiculosum* bran-peat inoculum was mixed into a peat-perlite planting mix at a concentration of 0.7%.

[y] Each individual shoot growth or root weight value is the mean of five replicate plants. * = two means within a row are significantly different at $P \leq 0.05$, according to the *F* test of the single degree of freedom contrasts from the analysis of variance.

[z] Means within a row followed by the same letter are not significantly different at $P \leq 0.05$, according to the *F* test from the analysis of variance.

TABLE 2. New shoot growth and the number of white root tips of sweet orange seedlings in the presence of *Penicillium funiculosum* (Pf) and two *Phytophthora* spp. at 8 weeks[x]

	New shoot growth (mm)[y]			White root tips (no.)		
Phytophthora sp.	No Pf	With Pf	Mean	No Pf	With Pf	Mean
None	52.4	81.6	67.0	28.4	20.0	24.2
P. citrophthora	32.4	96.0*	64.2	8.2	27.6	17.9
P. parasitica	49.0	98.8	73.9	24.4	34.4	29.4
Mean[z]	44.6 b	92.1 a		20.3	27.3	

[x] *P. funiculosum* bran-peat inoculum was mixed into a peat-perlite planting mix at a concentration of 0.7%.

[y] Each individual shoot growth or white root tip value is the mean of five replicate plants. * = two means within a row are significantly different at $P \leq 0.05$, according to the *F* test of the single degree of freedom contrasts from the analysis of variance.

[z] Means within a row followed by the same letter are not significantly different at $P \leq 0.05$, according to the *F* test from the analysis of variance.

T327S showed the greatest effect. The analysis of variance revealed that both *P. funiculosum* and *Phytophthora* spp. had a significant main effect on the growth of sweet orange seedlings ($P = 0.001$). However, there was no significant interaction between the *P. funiculosum* subisolates and *Phytophthora* spp. Both *Phytophthora* spp. significantly reduced plant growth in this experiment (Table 3). When the main effects of the three *P. funiculosum* subisolates were compared, it was shown that T327S caused a significant increase in the growth of sweet orange seedlings followed by T327H, which caused only a slight but generally not significant growth increase (Table 3). Analysis based on single degree of freedom contrasts showed that T327S significantly increased shoot growth ($P = 0.001$) and top ($P = 0.003$) and root ($P = 0.001$) weights when compared with the no *P. funiculosum* control in the presence of *P. citrophthora* (Table 3). Growth increase in the presence of *P. parasitica*, however, was slight and not statistically significant. T327H was effective only in increasing root weight ($P = 0.033$) and only against *P. citrophthora* (Table 3). T327L was not effective in the presence of either *Phytophthora* species.

Population dynamics of *P. funiculosum* in the peat-perlite planting mix. The three *P. funiculosum* subisolates (T327H, T327L, and T327S) were each added to peat-perlite planting mix at a concentration of 0.7%, which resulted in initial populations of 2×10^8, 1.4×10^8, and 3.8×10^{11} ppg, respectively. After 4 weeks, populations of the three subisolates had risen to 1.4×10^9, 8×10^8, and 2.8×10^{12} ppg, respectively. This represented a fivefold to sevenfold increase in the populations of the three subisolates. By 6 weeks, populations had declined to about 5×10^8, 5.5×10^7, and 1.2×10^{12} ppg for T327H, T327L, and T327S, respectively. Populations of the three subisolates further decreased to 2×10^7, 7×10^5, and 4×10^8 ppg, respectively, by 8 weeks, and all the populations then remained at about 4×10^5 through 11 weeks. Similar trends were observed for populations of subisolate T327S when *P. parasitica* or *P. cinnamomi* was added to the planting mix, except that in the presence of *P. cinnamomi*, populations of T327S continued to increase from week 4 to week 6.

Comparison of inoculation methods for *P. funiculosum*. Two systems for delivery of the antagonist were compared: 1) mixing *P. funiculosum* bran-peat inoculum into the planting mix before the azalea plants were transplanted, as in many other experiments, and 2) dipping roots of azalea plants into a *P. funiculosum* spore suspension before transplantation into the planting mix. Incorporating *P. funiculosum* inoculum by mixing it into the planting mix significantly reduced azalea root rot caused by *P. parasitica*, as was found in previous experiments. The new shoot growth of the *P. funiculosum* + *Phytophthora* treatment was about eight times greater than that of the *Phytophthora* alone treatment. However, disease was not reduced by *P. funiculosum* when the root dip method was used. With *P. cinnamomi* as the test pathogen, *P. funiculosum* slightly reduced azalea root rot when the inoculum was mixed into the planting mix. Again, there was no disease control when *P. funiculosum* was introduced by dipping roots into a spore suspension.

Effect of *P. funiculosum* inoculum concentration and additional inoculum on Phytophthora root rot. The bran-peat inoculum of *P. funiculosum* (T327S) was mixed with noninoculated bran-peat to reach dilutions of 1/10, 1/3, and 1/1 (undiluted) before it was incorporated into the peat-perlite planting mix. With the noninoculated bran-peat, this provided *P. funiculosum* inoculum concentrations of 0, 0.07%, 0.23%, and 0.7% in the planting mix. The analysis of a greenhouse experiment with *P. parasitica* showed that there was a significant effect of *P. funiculosum* concentration on the shoot growth ($P = 0.044$) and top ($P = 0.002$) and root ($P = 0.002$) weights of azalea, but *Phytophthora* had no significant effect and there was no significant interaction between *Phytophthora* and *P. funiculosum* concentration. *P. funiculosum* caused significant increases in plant growth regardless of the concentration of *P. funiculosum* used, even at 1/10 dilution (Table 4).

P. funiculosum inoculum grown in bran-peat was applied to the planting mix once or twice during a period of 6 or 12 weeks at a concentration of 0.35%, and a comparison of disease suppression of azalea root rot, caused by *P. cinnamomi,* was made. The experiment had the following three treatments based on application frequency: 1) *P. funiculosum* was applied once, and test plants were grown in 10-cm pots for 6 weeks before harvest; 2) *P. funiculosum* was applied once, and test plants were grown in 10-cm pots for 12 weeks before harvest; and 3) *P. funiculosum* at 0.35% was applied twice by transferring each test plant at 6 weeks from the original 10-cm pot to a 15-cm pot with additional

TABLE 3. New shoot growth and top and root weights of sweet orange seedlings in the presence of three *Penicillium funiculosum* (Pf) subisolates and two *Phytophthora* spp. at 8 weeks[v]

Measurement *Phytophthora* sp.	No Pf	T327H	T327L	T327S	Mean[w]
New shoot growth (mm)					
None	82.2[x]	114.0	63.4	110.2	92.4 a
P. citrophthora	22.0	54.2	44.6	86.0*[y]	51.7 b
P. parasitica	62.0	64.0	52.0	85.6	65.9 b
Mean[z]	55.4 bc	77.4 ab	53.3 c	93.9 a	
Top weight (mg)					
None	918.7	1,224.4	733.7	1,348.4*	1,056.3 a
P. citrophthora	414.1	660.8	526.7	1,027.8*	657.3 b
P. parasitica	689.8	739.0	600.7	962.1	747.9 b
Mean	674.2 bc	874.7 b	620.4 c	1,112.8 a	
Root weight (mg)					
None	324.4	394.5	297.0	421.6	359.4 a
P. citrophthora	126.5	254.7*	189.5	334.2*	226.2 c
P. parasitica	274.5	306.6	238.5	344.3	291.0 b
Mean	241.8 b	318.6 a	241.6 b	366.7 a	

[v] *P. funiculosum* bran-peat inoculum was mixed into a peat-perlite planting mix at a concentration of 0.7%.

[w] Each shoot growth and weight value is a mean of four *P. funiculosum* treatments with five replicate plants each. Means within a column followed by the same letter are not significantly different at $P \leq 0.05$, according to Fisher's LSD test.

[x] Each individual shoot growth or weight value is a mean of five replicate plants.

[y] * = subisolate means within a row are significantly different ($P \leq 0.05$) from that of the no *P. funiculosum* treatment, according to the *F* test of the single degree of freedom contrasts from the analysis of variance.

[z] Each shoot growth and weight value is a mean of three *Phytophthora* treatments with five replicate plants each. Means within a row followed by the same letter are not significantly different at $P \leq 0.05$, according to Fisher's LSD test.

fresh planting mix containing the *P. funiculosum* inoculum, and then plants were harvested at 12 weeks. The usual four treatments (control, *Phytophthora* alone, *P. funiculosum* alone, and *P. funiculosum* + *Phytophthora*) were included within each of the three frequency treatments. *P. funiculosum* at 0.35% did not improve azalea growth significantly when the plants were harvested at 6 weeks; however, new shoot growth of the *P. funiculosum* + *Phytophthora* treatment was almost as good (98%) as that of the noninoculated control (50 versus 51 mm). When the plants were harvested at 12 weeks, new shoot growth of the *P. funiculosum* + *Phytophthora* treatment was only 72% that of the noninoculated control (120 versus 166 mm). When *P. funiculosum* was applied twice over the 12-week period (each time at 0.35%), disease control was greatly improved. The new shoot growth and root weight of the *P. funiculosum* + *Phytophthora* treatment was not only significantly greater than those of the *Phytophthora* alone treatment, they were also greater than those of the noninoculated control. The analysis of variance showed that the effects of *P. funiculosum* and *P. cinnamomi* on azalea growth were both significant ($P = 0.001$). Both shoot growth and root weight increased significantly in the presence of *P. funiculosum* (Table 5). There was, however, no significant interaction between the *P. funiculosum* and *Phytophthora* treatments. The main effect of application frequency of *P. funiculosum* was also significant for both shoot growth and root weight. There was also a significant interaction between *P. funiculosum* application frequency and *P. funiculosum* ($P = 0.032$) or *Phytophthora* ($P = 0.004$) but not with both together. When the treatments of *P. funiculosum* + *Phytophthora* and *Phytophthora* alone were compared by single degree of freedom contrasts, the increases in new shoot growth and root weight for the twice-in-12-weeks treatment were both significant when *P. funiculosum* was present (Table 5). However, for the once-in-12-weeks treatment, only the increase in new shoot growth was significant. No significant differences in growth were observed for the once-in-6-weeks treatment.

TABLE 4. New shoot growth and top and root weights of azaleas in the presence of *Phytophthora parasitica* and four concentrations of *Penicillium funiculosum* at 8 weeks[w]

	New shoot growth (mm)	Top weight (g)	Root weight (mg)
P. parasitica[x]			
No	134.4	1.71	370.3
Yes	117.9	1.70	362.0
P. funiculosum concentration[y]			
0	82.6 b[z]	1.32 b	248.0 b
1/10	153.1 a	1.90 a	411.9 a
1/3	125.8 ab	1.71 a	371.9 a
1/1	143.0 a	1.89 a	432.9 a

[w] *P. funiculosum* bran-peat inoculum was mixed into a peat-perlite planting mix at a concentration of 0.7% without dilution (1/1) or diluted 1/3 or 1/10 with noninoculated bran-peat mixture.
[x] Each shoot growth and weight value is a mean of four *P. funiculosum* treatments with five replicate plants each.
[y] Each shoot growth and weight value is a mean of two *Phytophthora* treatments with five replicate plants each.
[z] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$, according to Fisher's LSD test.

Reduction of Phytophthora root rot by *P. funiculosum* in other planting mixes. The efficacy of *P. funiculosum* to reduce sweet orange root rot caused by *P. citrophthora* was tested in four planting mixes: peat-perlite (P-P) as in all previous experiments, peat-vermiculite (P-V), peat-sand (P-S), and pine bark-sand (or pine bark-peat-sand-perlite) (PB-S). Sweet orange seedlings grew better in the P-P and P-V mixes than in the P-S and PB-S mixes. Sweet orange grown in the two pine bark-containing mixes exhibited stunted growth and yellowing in all treatments. Regardless of the planting mix used, the presence of *P. funiculosum* improved the growth of sweet orange seedlings. The analysis of variance revealed that the main effects of *P. funiculosum* ($P = 0.001$), *Phytophthora* ($P = 0.001$), and mix ($P = 0.001$) were all significant in both experiments, as were the interactions of *P. funiculosum* × mix ($P = 0.001$) and *Phytophthora* × mix ($P = 0.004$) in one experiment. In both experiments, new shoot growth of sweet orange seedlings was increased as a result of the presence of *P. funiculosum* and was reduced as a result of the presence of *P. citrophthora* (Table 6). On the basis of single degree of freedom contrasts for the effect of *P. funiculosum* versus no *P. funiculosum* in each planting mix, significant growth increases attributable to *P. funiculosum* were consistently observed in P-P and P-V mixes (Table 6). Regarding biocontrol by *P. funiculosum*, on the basis of single degree of freedom contrasts for the effect of *P. funiculosum* + *Phytophthora* versus

TABLE 5. Effect of *Penicillium funiculosum* (Pf), *Phytophthora cinnamomi*, and the frequency of application of *P. funiculosum* on new shoot growth and root weight of azaleas[r]

Measurement / Frequency of application	Mean[s]	*P. funiculosum*[t]		*P. cinnamomi*			
		No	Yes	No[u]	Yes	No Pf[v]	With Pf
New shoot growth (mm)							
Once in 6 weeks	48.8 c[w]	42.0	55.5	55.5	42.0	37.0	47.0
Once in 12 weeks	140.5 b	116.0	165.0*[x]	188.0	93.0*	66.0	120.0*
Twice in 12 weeks	168.0 a	125.0	211.0*	175.5	160.5	101.0	220.0*
Mean[y]		94.3 b[z]	143.8 a	139.7 a	98.5 b		
Root weight (g)							
Once in 6 weeks	0.14 b	0.13	0.16	0.16	0.13	0.10	0.16
Once in 12 weeks	0.46 a	0.45	0.46	0.63	0.28*	0.24	0.32
Twice in 12 weeks	0.54 a	0.41	0.68*	0.58	0.51	0.36	0.66*
Mean		0.33 b	0.43 a	0.46 a	0.31 b		

[r] *P. funiculosum* bran-peat inoculum was mixed into a peat-perlite planting mix at a concentration of 0.35% at each application.
[s] Each shoot growth and root weight value is the mean of two *P. cinnamomi* and two *P. funiculosum* treatments with five replicate plants each.
[t] Each shoot growth and root weight value is the mean of two *P. cinnamomi* treatments with five replicate plants each.
[u] Each shoot growth and root weight value is the mean of two *P. funiculosum* treatments with five replicate plants each.
[v] Each shoot growth and root weight value is the mean of five replicate plants with or without *P. funiculosum*, both in the presence of *P. cinnamomi*.
[w] Means within the column followed by the same letter are not significantly different at $P \leq 0.05$, according to Fisher's LSD test.
[x] * = for each pair of columns, the two means within the row are significantly different at $P \leq 0.05$, according to the F test of the single degree of freedom contrasts from the analysis of variance.
[y] Each shoot growth and root weight value is the mean of two *P. cinnamomi* treatments or two *P. funiculosum* treatments and three application frequencies with five replicate plants each.
[z] For each factor, the two means within the row followed by the same letter are not significantly different at $P \leq 0.05$, according to the F test from the analysis of variance.

Phytophthora alone in each mix, significant growth increases were observed for P-V and P-S (and marginally significant increases for P-P) in the first experiment and for P-V and P-P in the repeat experiment (Table 6). Root rot reduction attributable to *P. funiculosum* was poorest in the pine bark-containing mixes in both experiments (Table 6).

The pH of P-P, P-V, P-S, and PB-S planting mixes increased from the initial values of 4.3–4.5, 4.5–4.7, 6.2–6.3, and 5.5–6.0 to 4.8–4.9, 5.1–5.5, 6.9–7.0, and 6.3–6.7, respectively, at the time of harvest at 6 weeks.

DISCUSSION

In our study, *P. funiculosum* was evaluated for its ability to suppress Phytophthora root rots of azalea and sweet orange by measuring mainly the increased shoot and root growth of the test plants. For experiments on sweet orange, recordings of the number of white root tips were also made. Although we took root rot ratings at the end of the experiments on azaleas, the ratings were so subjective and prone to bias and error that the data were considered not reliable and therefore were not used as results in this paper. Increased growth of test plants, in the presence of *Phytophthora*, as the result of treatments with *P. funiculosum* was interpreted as a suppression of Phytophthora root rot by the biocontrol agent.

Of the three subisolates of *P. funiculosum* evaluated, T327S was more effective than either T327H or T327L in suppressing Phytophthora root rot. The potential of subisolate T327S to serve as a biological control agent was demonstrated previously (17,49). In the present study, it effectively suppressed azalea root rot caused by *P. parasitica* but was only partially successful against *P. cinnamomi*. It also effectively suppressed sweet orange root rot caused by *P. citrophthora* but was less effective against *P. parasitica*. Variation in effectiveness among isolates of biocontrol agents has been previously documented (4,25). Ghisalberti et al (21) reported that the variability in the effectiveness of *Trichoderma harzianum* isolates as biocontrol agents probably resulted from the type and amount of antibiotics produced by each isolate. One possible reason for the difference in the effectiveness of suppression of Phytophthora root rot by the *P. funiculosum* subisolates is that the populations of T327S that developed in the planting mix were greater than those of either of the other two subisolates. This larger population may have been more effective in suppressing the *Phytophthora* inoculum. Another reason could be that the ability of T327S to produce antibiotics and enzymes was greater than that of either T327H or T327L (16). T327S was also more efficient in lysing *Phytophthora* mycelia than either of the other two subisolates (16).

The ability of introduced antagonistic fungi to establish and proliferate in the soil or other substrates is an important factor in successful biological control (31). The populations of the three subisolates of *P. funiculosum* increased five to seven times during the first 4 weeks in the planting mix. The proliferation of *P. funiculosum* in peat-perlite planting mix is likely the result of the availability of wheat bran as a food base. Wheat bran has been used by other workers (24,31,32,41,42) and has proved to be an effective food base for supporting antagonists in soils or planting mixes. The decline in the populations of *P. funiculosum* at 6 to 8 weeks could be caused by depletion of the food base. The proliferation of the antagonists may also depend on the age of the bran-peat inoculum used. Lewis and Papavizas (31) reported that populations of *Trichoderma viride* did not increase in soil when a 15- to 40-day-old bran preparation was used but did increase when a 1- to 3-day-old bran inoculum was used. Our results showed that the populations of *P. funiculosum* increased in the planting mix when 16- to 21-day-old bran preparations were used. The difference between our results and theirs is probably attributable to the use of different antagonists.

Dipping roots into a spore suspension of *P. funiculosum* was not an effective method for the delivery of the antagonist. One possible reason for this was the lack of a food base in the planting mix that could sustain increased growth of the antagonist. Another possible reason was that the spore suspensions used as inocula contained less than 5×10^7 spores per ml. This was quite low compared with the bran-peat inoculum that provided 10^9 to 10^{12} propagules per gram of *P. funiculosum* in the planting mix. The use of a higher concentration of spore suspension with the root dip method might improve the results but perhaps only slightly because of a lack of food base.

The efficacy of *P. funiculosum* to suppress azalea root rot,

TABLE 6. Effect of *Penicillium funiculosum* (Pf) and *Phytophthora citrophthora* on new shoot growth (mm) of sweet orange seedlings in four planting mixes at 6 weeks[r]

Experiment / Planting mix	Mean[s]	*P. funiculosum*[t]		*P. citrophthora*			
		No	Yes	No[u]	Yes	No Pf[v]	With Pf
Experiment 1							
Peat-perlite	39.8 a[w]	34.0	45.6*[x]	43.6	36.0	29.6	42.4
Peat-vermiculite	36.3 a	26.1	46.5*	42.6	30.0*	18.8	41.2*
Peat-sand	22.6 b	13.3	32.0*	27.3	18.0	1.0	35.0*
Pine bark-sand	7.6 c	5.8	9.3	11.6	3.5	2.0	5.0
Mean[y]		19.8 b[z]	33.4 a	31.3 a	21.9 b		
Experiment 2							
Peat-perlite	43.6 b	35.7	51.5*	50.1	37.1*	26.6	47.6*
Peat-vermiculite	52.5 a	36.2	68.8*	69.7	35.3*	19.8	50.8*
Peat-sand	15.4 c	12.3	18.6	18.4	12.5	6.6	18.4
Pine bark-sand	16.7 c	15.8	17.6	25.0	8.4*	6.8	10.0
Mean		25.0 b	39.1 a	40.8 a	23.3 b		

[r] *P. funiculosum* bran-peat inoculum was mixed into each of the four planting mixes at a concentration of 0.7%.
[s] Each shoot growth value is the mean of two *P. funiculosum* treatments and two *P. citrophthora* treatments with five replicate plants each.
[t] Each shoot growth value is the mean of two *P. citrophthora* treatments with five replicate plants each.
[u] Each shoot growth value is the mean of two *P. funiculosum* treatments with five replicate plants each.
[v] Each shoot growth value is the mean of five replicate plants with or without *P. funiculosum*, both in the presence of *P. citrophthora*.
[w] Means within the column followed by the same letter are not significantly different at $P \leq 0.05$, according to Fisher's LSD test.
[x] * = for each pair of columns, the two means within the row are significantly different at $P \leq 0.05$, according to the F test of the single degree of freedom contrasts from the analysis of variance.
[y] Each shoot growth value is the mean of two *P. citrophthora* treatments or two *P. funiculosum* treatments and four planting mixes with five replicate plants each.
[z] For each factor, the two means within the row followed by the same letter are not significantly different at $P \leq 0.05$, according to the F test from the analysis of variance.

caused by *P. cinnamomi*, decreased with time. *P. funiculosum* at 0.35% concentration was not effective over a 12-week period. Data showed that the decline in population of *P. funiculosum* in the planting mix occurred by 8 weeks. The population of *P. funiculosum* may have continued to decline in the planting mix over a 12-week period, which would account for its lowered efficiency. However, effective disease suppression was achieved after the original 6-week incubation period by retransplanting the test plants into new planting mix that had been freshly amended with *P. funiculosum*. This provides a way to reapply *P. funiculosum* inoculum to the planting mix in nursery containers and thus prolong disease suppression by the biocontrol agent. In commercial nurseries, rooted azalea cuttings are transplanted to progressively larger containers twice a year. These two transplantings would be the ideal time for the incorporation of *P. funiculosum* inoculum into the planting mix.

Ideally, assessment of biocontrol capability should be done in an environment as similar as possible to the environment where the antagonist will be applied. If the target organism is a pathogen of potted plants in greenhouse production, then assessment in a greenhouse is an appropriate environment, and suitable soilless potting mixes should be used (33). The selection of a planting mix is one of the most important decisions in the production of container-grown ornamental plants. Five common materials have been used routinely in recent years in the commercial preparation of planting mixes: peat moss, sawdust, vermiculite, perlite, and sand (10). Our work has shown that *P. funiculosum* can suppress Phytophthora root rots in several different planting mixes composed of peat, perlite, vermiculite, and sand in various proportions. It is likely that *P. funiculosum* may also be effective in many other planting mixes used by commercial nurseries.

An advantage of using peat-perlite or peat-vermiculite as planting mixes is the initial low pH of such mixes; the pH levels of these two mixes were 4.3 to 4.5 and 4.5 to 4.7, respectively. The low pH is of value in controlling Phytophthora root rot of plants, such as azaleas, that are tolerant of low pH. The use of low pH and application of sulfur to reduce soil pH have been effective in controlling a number of Phytophthora diseases (36,39). Low pH may adversely affect the activity of pathogens such as *Phytophthora* spp. (39) but stimulate the growth and enhance the activity of the hyphomycetous antagonist, such as *P. funiculosum*.

Both azalea and sweet orange grown in the planting mixes amended with *P. funiculosum* alone grew faster and better than those in the nonamended mixes. This phenomenon was similar to that reported for *Trichoderma* spp., another hyphomycetous biocontrol group that produces a pronounced increased growth of many plants tested (2,12). Further studies on increased growth of these plants and the mechanisms responsible for the increased growth responses provided by *P. funiculosum* have been conducted in the greenhouse and nursery (16). These results will be reported in detail elsewhere. Integration of *P. funiculosum* with metalaxyl, an Oomycetes-specific fungicide, and mechanisms of biological control by *P. funiculosum* have also been studied (16,18), and the results in detail will be reported in the future.

Subisolate T327S of *P. funiculosum* has been deposited at the American Type Culture Collection, Rockville, Maryland, and was assigned accession number ATCC 96014.

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