Evaluation of *Pythium nunn* as a Potential Biocontrol Agent Against Phytophthora Root Rots of Azalea and Sweet Orange

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ABSTRACT

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Pythium nunn parasitized the hyphae, sporangia, chlamydospores, and sexual organs of five isolates of *P. cinnamomi*, *P. citrophthora*, and *P. parasitica* in vitro, and caused inhibition of mycelial growth of these isolates. Population densities of *P. nunn* in a peat/sand mix, monitored up to 8 wk, declined gradually unless 1% ground rolled oats were added to the mix at 2 wk. Population densities of all three *Phytophthora* spp. also increased after 1% ground rolled oats were added. Population densities of *P. cinnamomi*, *P. citrophthora*, and one isolate of *P. parasitica* in oat-amended treatments were reduced in the presence of *P. nunn*, but no reduction in population densities of the other isolate of *P. parasitica* occurred in the presence of *P. nunn*, with or without oats. The effectiveness of *P. nunn* in suppressing root rot of azalea (*Rhododendron* spp.) caused by *P. cinnamomi* or *P. parasitica*, and root rot of sweet orange (*Citrus sinensis*) caused by *P. parasitica*, was evaluated in the peat/sand mix amended with 1% ground rolled oats in greenhouse tests. *P. nunn* at 300 propagules per gram did not suppress azalea or sweet orange root rot. At 1,000 propagules per gram, it significantly suppressed sweet orange root rot caused by *P. parasitica*. *P. nunn* did not affect the growth of azalea but slightly reduced sweet orange seedling growth.

Additional keyword: antagonism.

Phytophthora root rot is a major disease of woody perennials including azalea (5,13) and citrus (28). Control methods such as preplant soil fumigation and use of resistant varieties have not proved totally effective. Chemicals, such as metalaxyl and fosetyl aluminum, can produce good disease suppression (3,4, 7,11), but are not always desirable due to high costs, probability of development of resistant strains, and potential hazards to the environment. Biological methods for controlling Phytophthora root rot have therefore been sought by researchers in recent years (6,9,12,19,25–27,33).

Pythium nunn Lifshitz, Stanghellini & Baker is a mycoparasite (15) first found in a soil in Colorado (17). It is known to efficiently suppress preemergence damping-off of cucumber seedlings caused by *Pythium ultimum* in numerous greenhouse tests (16,20–22,24). It is also antagonistic in vitro to several other root disease fungi (8,14,15) including two *Phytophthora* spp. (15), and is nonpathogenic to all experimental plants so far tested (16). Its antagonistic activity against host fungi included coiling around, penetration, and lysis of host hyphae or reproductive structures (15,16). *Pythium nunn* controls *P. ultimum* on cucumber by reducing the population density of the latter (21), thus decreasing the incidence of damping-off. Various organic substrates including oatmeal, when used as soil amendment, greatly increased *P. nunn* populations saprophytically in soil (21,22). *Pythium nunn* can efficiently compete with *P. ultimum* for available organic substrates, thereby reducing the saprophytic activity and inoculum potential of the pathogen in soil (21–23).

The purpose of this research was to study the antagonism of *Pythium nunn* to three *Phytophthora* spp. in vitro, to monitor the population dynamics of *P. nunn* and the three *Phytophthora* spp. in a planting mix, and to evaluate the efficiency of *P. nunn* as a biocontrol agent against Phytophthora root rots of two woody perennials, azalea and sweet orange. A portion of the work has been reported briefly (10).

MATERIALS AND METHODS

Fungal isolates. Five isolates of three *Phytophthora* spp. were used in the studies: *P. cinnamomi* Rands, isolates T139 (= Pc 40, ex avocado) and B101 (= T602, ex azalea); *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian, isolate P1156 (= T590, ex citrus); and *P. parasitica* Dast. (syn. *P. nicotianae* Breda de Haan), isolates T131 (ex citrus) and T593 (ex azalea). Isolate N2 of *Pythium nunn* was provided by R. Baker.

Test plants. Rooted cuttings of azalea (hybrid of *Rhododendron* spp.) cv. Chimes were provided by Monrovia Nursery (Azusa, CA). The rooted cuttings used in the tests were 2–6 mo old, and had been grown in flats in a peat/perlite (7:3, v/v) planting mix, under natural light, at a temperature of 24 ± 5 C. Seeds of sweet orange (*Citrus sinensis* (L.) Osbeck 'Madam Vinus') were planted in flats in a peat/sand (1:1, v/v) planting mix (U.C. mix formulation 5). Seedlings used in the tests were 2–3 mo old.

Culture media. All fungi were cultured and maintained on cleared V8 (V8C) agar (20% Campbell V8 juice, 0.2% $CaCO_3$, clarified by centrifugation, 1.5% agar). The hyphal interaction studies were performed on V8C agar or 2% water agar. Chlamydospores of *P. cinnamomi* and *P. parasitica* were produced in cleared V8 broth, which had the same ingredients as V8C agar except that the agar was omitted and V8 juice was reduced to 10%.

In vitro interactions between *Pythium nunn* and *Phytophthora* spp. Disks (4-mm-diameter) cut with a cork borer from the margins of actively growing colonies of each isolate of the three *Phytophthora* spp. were transferred to the edge of water-agar plates and incubated at 25 C in the dark. After 3 days, two 1.2-mm-diameter disks of *P. nunn* cut with a cannula were added to the plate approximately 1 mm from each side of the *Phytophthora* disk and directly on the surface of existing *Phytophthora* mycelia. Control plates containing *Phytophthora* received 1.2-mm disks of agar medium only. After 2 days of incubation, morphological changes in the areas of interaction between *Pythium* and *Phytophthora* were observed at 1-day intervals for 5 days using

a light microscope. This method was also used in the study of inhibition of mycelial growth of the *Phytophthora* spp., except that V8C agar was used and *Phytophthora* was allowed to grow for only 2 days before *P. nunn* was added. The linear growth of *Phytophthora* in the *Pythium* and control treatments was measured after 7 days of incubation.

Parasitism of reproductive structures of *Phytophthora* by *P. nunn* was also studied. *Phytophthora* sporangia were produced on mycelial mats in 2 ml of water in 35-mm petri plates by the method described by Al-Hedaithy and Tsao (1). Two 1.2-mm disks of *P. nunn* were then placed near the margins of the mycelial mats containing the sporangia. Daily microscopic observations of the areas of interaction between the two were made for 2–3 days. Chlamydospores of *Phytophthora* were produced by a method (29) involving the incubation of submerged mycelial mats in deep water at 18 C. The mycelial mats containing chlamydospores, after being air-dried briefly and cut into small pieces, were added to the advancing colony margins of *P. nunn,* which had been grown for 2 days on water-agar plates. Observations were made daily for 2–5 days. Sexual structures of *P. cinnamomi* and *P. parasitica* were produced by pairing two isolates of opposite but compatible mating types, A^1 and A^2, on V8C-agar plates. The inocula of the two mating types were placed 30 mm apart near the center of the plate and incubated at 25 C in the dark. The two colonies came into contact after 2–3 days, and two 1.2-mm disks of *P. nunn* were added to the *Phytophthora* growth near the contact line 7 days after contact. Observations were made daily for 2–5 days.

Infestation of planting mix with *Pythium nunn* and *Phytophthora* isolates. Planting mix was infested with the fungal cultures for experiments on population dynamics and on root rot suppression in the greenhouse. The peat/sand planting mix consisted of 50% peat moss and 50% fine sand, plus 2.2 kg of dolomite, 1.5 kg of super phosphate, 148 g of KNO_3, and 148 g of K_2SO_4 per cubic meter of the mix. The mix had a pH of 5.5–6.0 and was a modification (formulation 5) of the U.C. mix (2). Before use, the mix was initially sterilized by steaming but was recolonized by airborne microbiota during storage.

Initial inocula of *P. nunn* and the five isolates of *Phytophthora* were prepared by adding five 4-mm disks obtained from the margins of an actively growing colony of either *Pythium* or *Phytophthora* isolate to each 100-mm glass petri plate containing 30 g of resterilized planting mix amended with 1% (w/w) ground rolled oats (Quaker Oats Co., Chicago, IL). Control plates received agar disks only. Resterilization was accomplished by autoclaving for 1 h on each of 2 consecutive days. The plates were incubated at 25 ± 1 C for 3 wk before the population density, expressed as propagules per gram (ppg), of each isolate in these initial inoculum plates was determined by dilution plating. Plating was done on the $P_{10}VP$ selective medium containing pimaricin, vancomycin, and pentachloronitrobenzene (32). These colonized mix preparations, alone or in combinations and at calculated amounts, were then used as the inocula to infest large quantities of the nonsterile mix to reach the desired concentrations, which were used in experiments on population dynamics and disease suppression. Thorough mixing was accomplished by rotating inflated plastic bags. The preparations contained 0, 50, and 200 ppg or 0, 30 and 300 ppg of each *Phytophthora* isolate, depending on the experiment. Each preparation further contained 0, 300 or 1,000 ppg of *P. nunn*. After 14 days, each preparation was separated into two equal portions. One portion was amended with 1% ground rolled oats and the second portion, which was not amended, served as the control. All preparations were incubated at 25 ± 1 C in large plastic bags, which retain the moisture but allow gas exchange, and were kept in 3-L open plastic pots.

Quantitation of *Pythium* and *Phytophthora* population densities in the infested planting mix. Population densities of *P. nunn* were determined by dilution plating on the $P_{10}VP$ medium (32), and the *Phytophthora* spp. on the PVPH medium (31). The latter contained the same ingredients as $P_{10}VP$ except for the addition of hymexazol at 50 mg/L, which inhibits the growth of *P. nunn*. Population assays were made weekly or biweekly. Appropriate dilutions were prepared so as to attain about 20–30 colonies per plate. The different *Phytophthora* spp. and *P. nunn* were distinguishable from each other macroscopically and microscopically, based on characteristic differences in colony and hyphal morphology of these species.

Greenhouse experiments on root rot suppression. The biocontrol efficiency of *P. nunn* against root rot of azalea caused by *P. cinnamomi* (isolate B101) and *P. parasitica* (T593) and root rot of sweet orange caused by *P. parasitica* (T131) was evaluated in greenhouse tests. The infested planting mix, which was initially prepared for population assays and had been amended with 1% ground rolled oats, was also used as the planting mix in the greenhouse experiments on disease suppression. Each oat-amended mix preparation was incubated in plastic bags, as described earlier, for an additional 2 wk before being transferred to each of five 10-cm square pots (800-ml volume), into which an azalea rooted cutting or sweet orange seedling was transplanted. A 15-cm-diameter saucer was placed under each pot and the plants in the greenhouse were watered with complete Hoaglund solution. All pots received weekly heavy watering ("waterlogging") (30) and the soil water matric potential was maintained at 0 to −10 kPa at all times. Each test plant was marked with indelible India ink at the shoot apex or apices and new shoot growth was measured periodically during an 8-wk period. Test plants were harvested at 8 wk; top and roots were separated and oven dried at 85 C for 24 h, and dry weights were recorded. For sweet orange seedlings, the number of white root tips were also counted after plants were harvested (30).

Statistical analyses. Three replicates were used in in vitro experiments on interactions and on population dynamics, each of which was repeated three times. Two-way analyses of variance were performed on the log-transformed population data, and mean separation was done using Duncan's multiple range test or Fisher's LSD test. Five single-plant replicates of each treatment were used in greenhouse experiments. Pots were arranged on the greenhouse benches in a randomized complete block design. Suppression of both azalea and citrus root rot was assessed in two greenhouse experiments, with a $2 \times 2 \times 3$ or a 2×3 factorial combination of treatments. The analyses of variance were calculated using MSTAT 4.0C (Michigan State University) and mean separation was done using Student's *t* test or Fisher's LSD test.

RESULTS

In vitro interactions between *Pythium nunn* and *Phytophthora* spp. Three types of hyphal interactions were observed between *P. nunn* and the five isolates of *Phytophthora* spp.: *1) P. nunn* hyphae coiled around the host hyphae. The coiling was either loose or massive. *2) P. nunn* penetrated the host hyphae. *3)* The host hyphae became lysed and disintegrated. The results are summarized in Table 1. In the areas of interaction on water-agar plates, *P. nunn* first grew toward, then massively coiled around, *P. cinnamomi* hyphae (Fig. 1A and E). Penetration and subsequent lysis of hyphae were observed in both isolates of *P. cinnamomi* (Fig. 1D and F). *P. nunn* acted differently on the two isolates of *P. parasitica*; it coiled massively around and penetrated hyphae of isolate T131 (Fig. 1C), but only coiled loosely around the hyphae of isolate T593 (Fig. 1B). This differing action of *P. nunn* on the two isolates was observed in each of the three experiments. *P. nunn* coiled loosely around hyphae of *P. citrophthora*, but penetration of hyphae was not observed in any of the three experiments.

Parasitism of the different spore structures of *Phytophthora* by *P. nunn* is summarized in Table 1. The sporangia of *P. citrophthora* and both isolates of *P. parasitica* were coiled and penetrated by *P. nunn* (Fig. 2A and B). However, sporangia of *P. cinnamomi* were rarely parasitized. The chlamydospores of *P. parasitica* were very susceptible to *P. nunn*; many of them were coiled (Fig. 2C), penetrated, and lysed. However, parasitism of the chlamydospores of *P. cinnamomi* by *P. nunn* was never observed in spite of careful, repeated, microscopic examination. *P. nunn* hyphae penetrated into antheridia and oogonia of both isolates of *P. parasitica*

(Fig. 2D) and finally lysed these structures. The antheridia and oogonia of both isolates of *P. cinnamomi* were penetrated by *P. nunn*, but were not lysed during the period of observation (Fig. 2E). No evidence was found that *P. nunn* penetrated the wall of mature oospores, but the contents of oospores were affected and appeared abnormal. The percentage of abnormal oospores in the areas of interaction was about 100–170% greater than in the areas without *P. nunn*.

Pythium nunn also inhibited mycelial growth of *Phytophthora* spp. on V8C-agar medium. Linear growth inhibition of *P. cinnamomi* and *P. parasitica* was considerable; the averages of the three experiments ranged from 28.8 to 48.1% (Table 1). However,

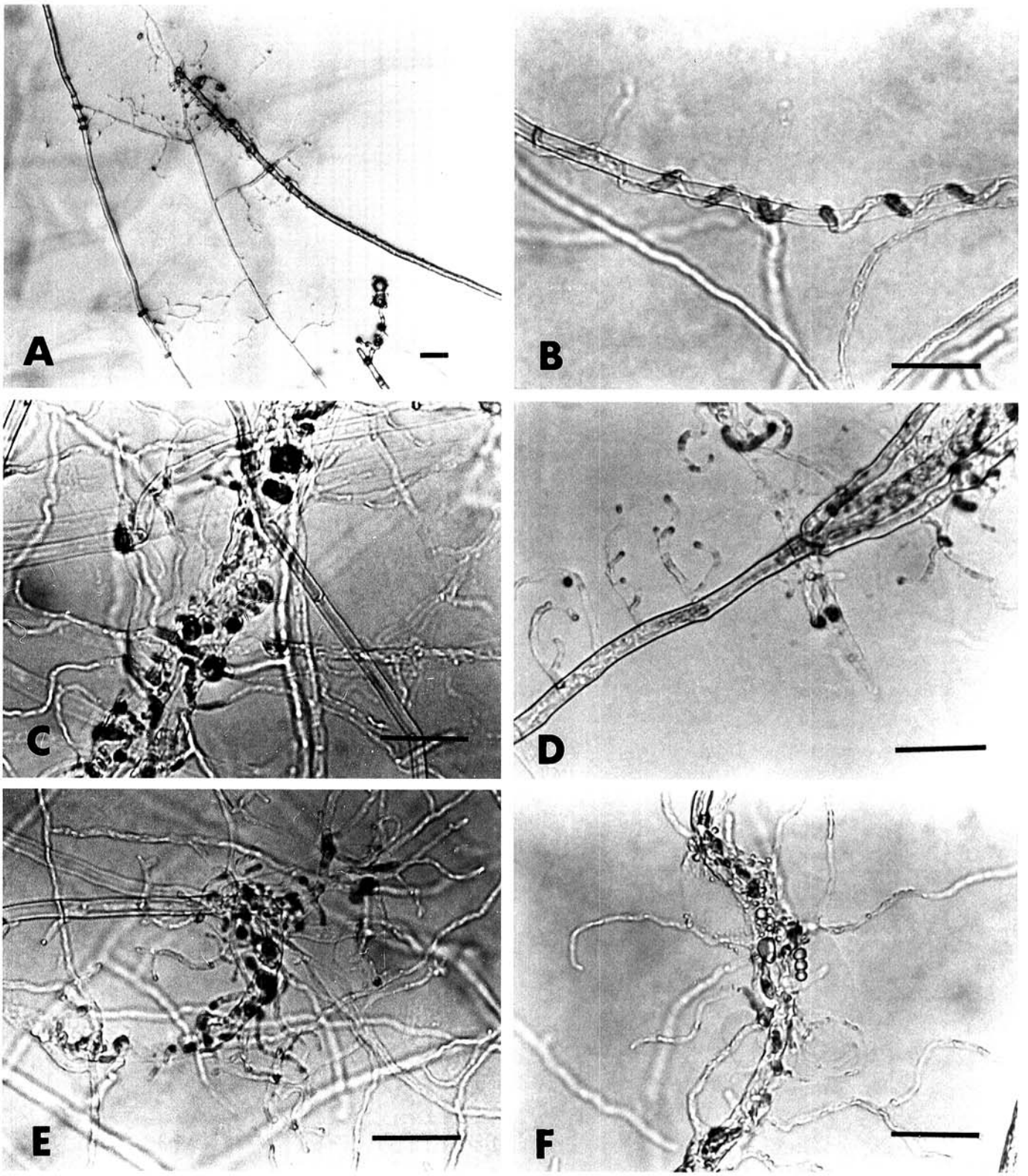

Fig. 1. Photomicrographs of hyphal interactions between *Pythium nunn* and *Phytophthora* spp. **A,** Directional growth of hyphal branches of *P. nunn* (narrower) toward hyphae of *P. cinnamomi* (T139) (broader) and coiling of *P. nunn* hyphae around host hyphae. **B,** Loose coiling of *P. nunn* hypha around a hypha of *P. parasitica* (T593). **C,** Massive coiling of *P. nunn* hyphae around a hypha of *P. parasitica* (T131). **D,** A *P. nunn* hypha growing inside hypha of *P. cinnamomi* (B101) and emerging from it. **E** and **F,** Hyphae of *P. cinnamomi* (T139) distorted and lysed by *P. nunn*. Bars = 30 μm.

only slight reduction in *P. citrophthora* growth was observed. Although linear growth of *P. citrophthora* mycelia on V8C agar was similar with or without *P. nunn*, the density of mycelial growth was drastically reduced in the presence of *P. nunn*. Similar results on inhibition of mycelial growth were also observed on water agar, but the growth of both *Pythium* and *Phytophthora* was very thin and could not be measured accurately.

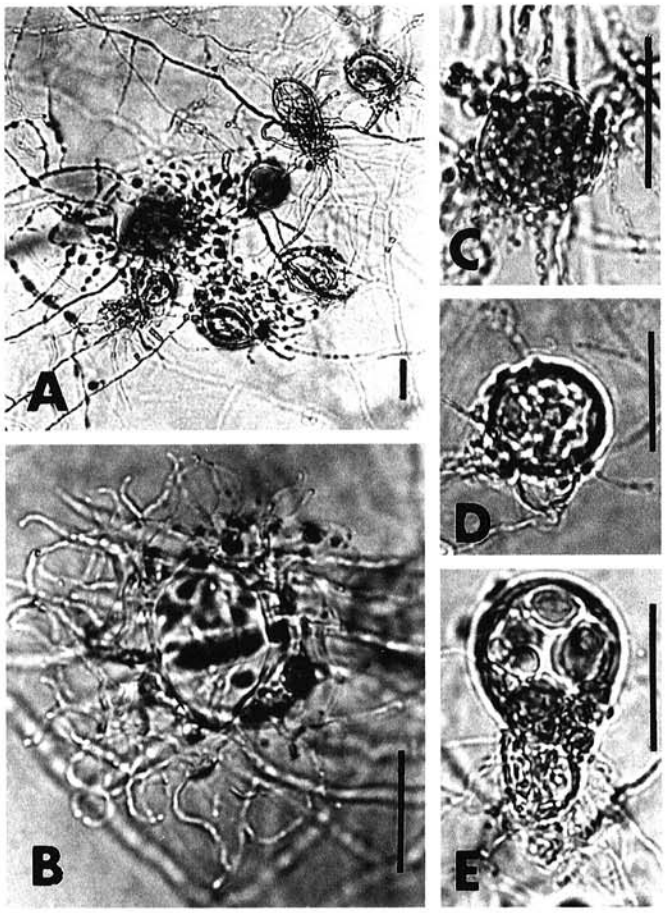

Fig. 2. Photomicrographs of parasitism of reproductive structures of *Phytophthora* spp. by hyphae of *Pythium nunn*. **A** and **B,** Sporangia of *P. citrophthora* coiled massively by hyphae of *P. nunn*. **C,** Massive coiling of hyphae of *P. nunn* around a chlamydospore of *P. parasitica* (T593). **D,** Hyphae of *P. nunn* penetrating into an oogonium of *P. parasitica* (T131). **E,** Antheridium of *P. cinnamomi* (T139) penetrated and colonized by *P. nunn*. Bar = 30 μm.

Changes in population densities of *Pythium nunn* and *Phytophthora* spp. in the presence of nutrients and in mixed populations. The population density of *P. nunn* when alone in the nonamended planting mix decreased gradually during the 8-wk incubation. When 1% ground rolled oats were added at 2 wk, the population density increased more than 40-fold by the sixth week (Fig. 3). In the presence of *P. parasitica, P. nunn* population density at 8 wk was about 80 times greater in the oat-amended treatment than in the nonamended treatment. Surprisingly, however, the population density of *P. nunn* was significantly less in the *Phytophthora*-infested mix than in the no-*Phytophthora* mix of the oat-amended treatments at 4 and 6 wk (Fig. 3).

For *P. parasitica* (isolate T131), the population density increased about three- to fivefold in the nonamended mix during the first 2 wk and remained at this level over the next 6 wk, but increased about 13- to 20-fold during this period after ground rolled oats were added (Fig. 4A). With an initial inoculum density at 200 ppg, population of isolate T131 in the mix was not affected in either treatment, with or without *P. nunn* (Fig. 4A). In a separate experiment, the pattern of population changes of *P. citrophthora* was slightly different from that of *P. parasitica*. Population densities of *P. citrophthora* decreased slightly (about twofold) in the

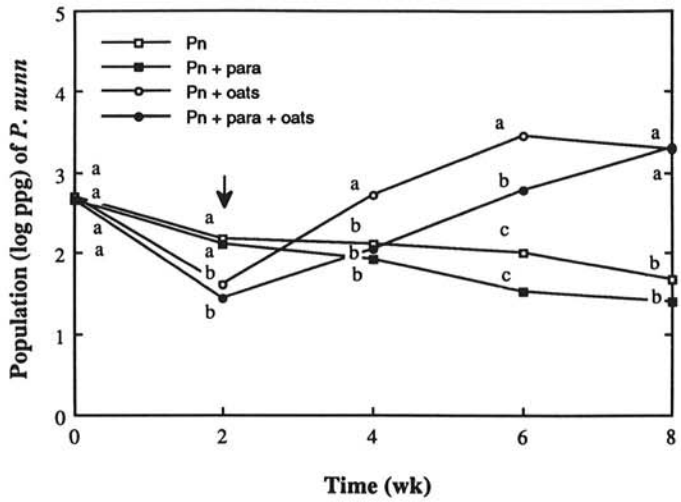

Fig. 3. Changes in population density (propagules per gram, ppg) of *Pythium nunn* (Pn) in planting mix with or without *Phytophthora parasitica* (para, T131). Sampling done biweekly. Initial inoculum density of *P. nunn,* 300 ppg; of *P. parasitica,* 50 ppg. Amendment of 1% ground rolled oats added at 2 wk (arrow). Values at a given sampling time with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 1. Parasitism and growth inhibition of *Phytophthora* species by *Pythium nunn* in vitro

	Phytophthora cinnamomi isolates		*Phytophthora citrophthora* isolate	*Phytophthora parasitica* isolates	
	T139	B101	P1156	T131	T593
Parasitism[y] of structures					
Hyphae	Massive coiling, penetration, lysis	Massive coiling, penetration, lysis	Loose coiling	Massive coiling, penetration	Loose coiling
Sporangia	Coiling rare, penetration	No interaction	Massive coiling, penetration	Massive coiling, penetration	Massive coiling, penetration
Chlamydospores	No interaction	No interaction	Not applicable	Coiling, penetration, lysis	Coiling, penetration, lysis
Antheridia & oogonia	Penetration	Penetration	Not applicable	Penetration, lysis	Penetration, lysis
Inhibition of linear growth (%)[z]					
Experiment 1	48.8	63.2	13.5	15.1	54.5
Experiment 2	55.7	35.5	0.8	36.3	58.4
Experiment 3	39.8	41.8	0.2	35.0	0.2

[y] Parasitism of hyphae, sporangia, chlamydospores, and sexual structures was studied on water agar, in water, on water agar, and on V8C agar, respectively. The results summarized are from three experiments.

[z] Linear mycelial growth of *Phytophthora* on V8C agar was measured 7 days after adding *P. nunn* inoculum. Percent reduction was calculated in comparison to growth on the control plates without *P. nunn*.

nonamended mix, with or without *P. nunn*. However, after ground rolled oats were added, population density increased up to 4 wk before decreasing (Fig. 4B). In oat-amended mix, *P. citrophthora* population densities at various times were significantly ($P = 0.05$) less in the presence of *P. nunn* than in its absence (Fig. 4B).

In another experiment, in which both *P. cinnamomi* (isolate B101) and *P. parasitica* (isolate T593) were used, changes in *Phytophthora* populations were monitored weekly for 4 wk in the mix that was amended with 1% ground rolled oats at the time of infestation (week 0). Population densities of both *Phytophthora* spp. increased in the oat-amended mix, but the increase was significantly ($P = 0.05$) less in the *P. nunn* treatments (Fig. 5).

Effect of *Pythium nunn* on Phytophthora root rots of azalea and sweet orange. Initial inoculum densities of 0 and 300 ppg of *P. nunn*, combined with 0, 30, and 300 ppg of *P. cinnamomi* (isolate B101) or of *P. parasitica* (isolate T593), were incorporated into the oat-amended planting mix to study the effectiveness of *P. nunn* on the suppression of root rot of azalea. The analysis of variance revealed that neither *P. nunn* nor the *Phytophthora* spp. had a significant effect on either top or root weights (Table 2). Only the initial *Phytophthora* inoculum density had a significant effect on plant growth. In this experiment, more severe disease was caused by *P. cinnamomi* than by *P. parasitica*, and both top and root weights decreased as the inoculum level was increased (Table 3). The data of the repeat experiment were similar; the azaleas again grew as poorly in the peat/sand mix as they had in the first experiment.

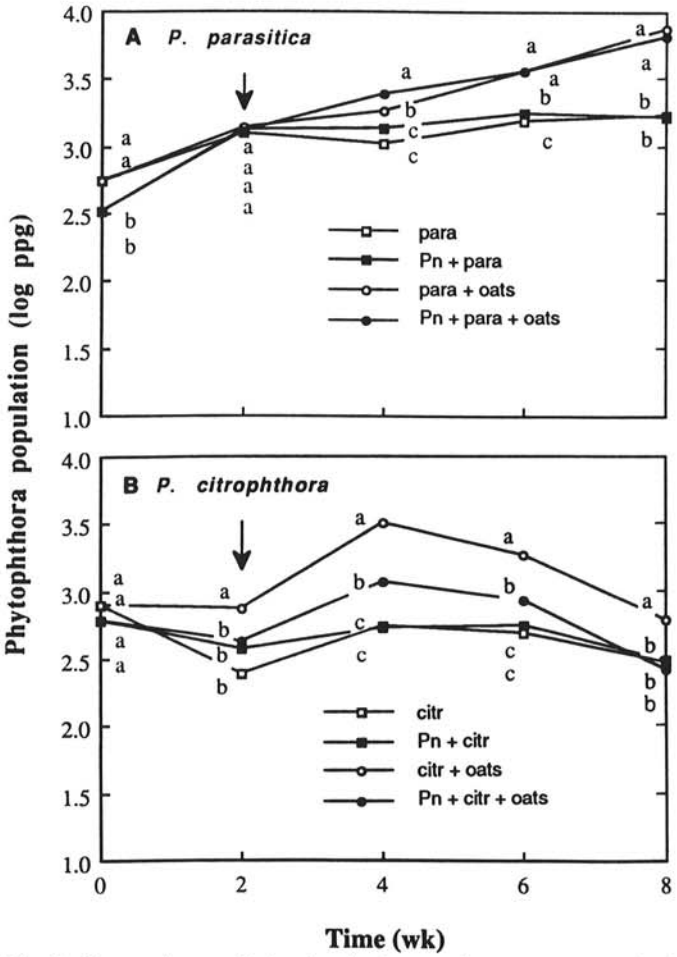

Fig. 4. Changes in population density (propagules per gram, ppg) of **A,** *Phytophthora parasitica* (para, T131) and **B,** *P. citrophthora* (citr, P1156) in the planting mix with or without *Pythium nunn* (Pn). Sampling done biweekly. Initial inoculum density of *P. parasitica,* 200 ppg; of *P. citrophthora,* 300 ppg; of *P. nunn,* 300 ppg. Amendment of 1% ground rolled oats added at 2 wk (arrow). Values at a given sampling time with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Two initial inoculum densities of *P. nunn*, 0 and 300 or 0 and 1,000 ppg, combined with 0, 50, and 200 ppg or 0, 30, and 300 ppg of *P. parasitica* (T131), were used to evaluate the effectiveness of *P. nunn* on the suppression of root rot of sweet orange in two separate experiments. *P. nunn* at the low inoculum density

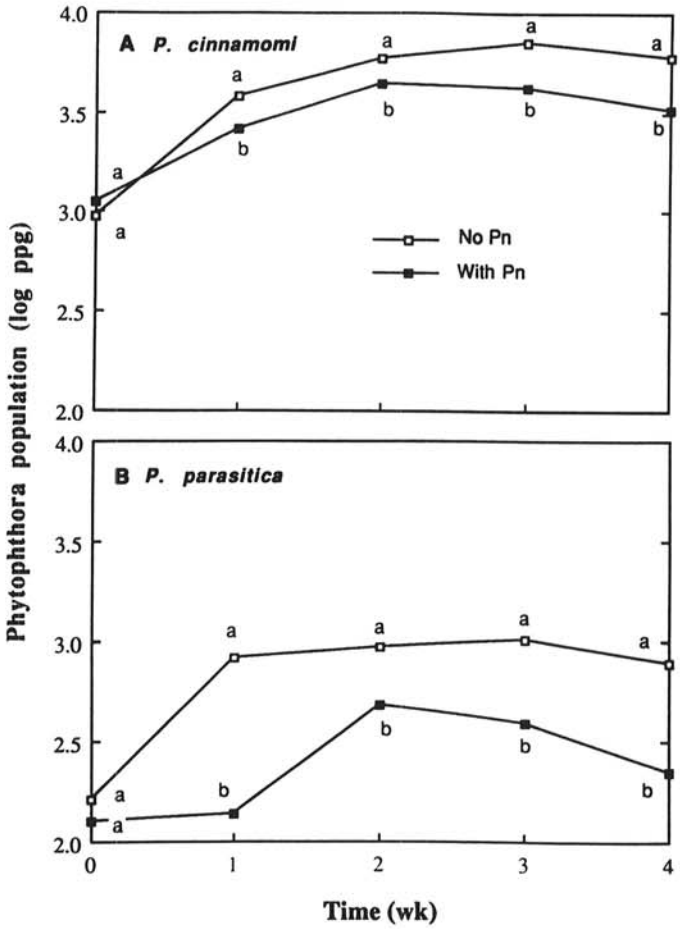

Fig. 5. Changes in population densisty (propagules per gram, ppg) of **A,** *Phytophthora cinnamomi* (B101) and **B,** *P. parasitica* (T593) in oat-amended planting mix, with or without *Pythium nunn* (Pn). Initial inoculum densities of each of the two *Phytophthora* spp. and *P. nunn* were 300 ppg. Amendment of 1% ground rolled oats added to all treatments at wk 0. Values at a given sampling time with the same letter are not significantly different ($P = 0.05$) according to Fisher's LSD test.

TABLE 2. Analysis of variance for the effects of *Pythium nunn* (Pn), two *Phytophthora* spp. (Phy), and three *Phytophthora* inoculum densities (ID) on azalea top and root weights[z]

Source of variation	df	Mean square	*F*	*P*
Top weight				
Pn	1	190.82	0.01	1.000
Phy	1	35,380.82	1.66	0.203
Pn × Phy	1	252.15	0.01	1.000
ID	2	107,449.32	5.05	0.010
Pn × ID	2	1,046.32	0.05	1.000
Phy × ID	2	11,455.82	0.54	1.000
Pn × Phy × ID	2	395.15	0.02	1.000
Error	48	21,268.48		
Root weight				
Pn	1	207.58	1.02	0.316
Phy	1	452.65	2.23	0.141
Pn × Phy	1	2.99	0.01	1.000
ID	2	744.26	3.67	0.032
Pn × ID	2	14.20	0.07	1.000
Phy × ID	2	150.98	0.74	1.000
Pn × Phy × ID	2	3.20	0.02	1.000
Error	48	202.84		

[z]See Table 3 for treatments.

(300 ppg) did not suppress sweet orange root rot; in fact, the plant growth was less in the *P. nunn* treatment (Tables 4 and 5). The effect of the *P. parasitica* inoculum density on plant growth was significant. When *P. nunn* was tested at 1,000 ppg, there was a significant interaction between *P. nunn* and the inoculum density of *P. parasitica*. The effect of *P. nunn* in the absence of *Phytophthora* was significant for new shoot growth but not for number of white root tips (Table 6). At *Phytophthora* inoculum density of 300 ppg, the effect of *P. nunn* at 1,000 ppg was significant for both new shoot growth and number of white root tips (Tables 6 and 7). However, the overall effect of *P. nunn* was not significant in either case (Table 6). The effect of *P. parasitica* inoculum density was again significant in both cases. Disease in the *Phytophthora*-alone (300 ppg) treatment was very severe. All five plants in this treatment died within 6 wk, but in the *P. nunn* + *Phytophthora* treatment only one out of the five plants was dead when the experiment was terminated at 8 wk (Fig. 6). At 1,000 ppg, however, the *P. nunn*-alone treatment reduced the new shoot growth of sweet orange seedlings to 60% of the no-*Phytophthora* no-*P. nunn* control (Table 7). The number of white root tips in the *P. nunn*-alone treatment was not greatly reduced, however.

DISCUSSION

Pronounced mycoparasitism by *P. nunn* on hyphae, sporangia, chlamydospores, and sexual organs of *P. cinnamomi*, *P. citrophthora*, and *P. parasitica* was observed in vitro. Parasitism of *P. cinnamomi* and *P. parasitica* by *P. nunn* has been reported previously by Lifshitz et al (15), who described a "slow reaction" between *P. nunn* and the two *Phytophthora* spp. but not the lysis phenomenon that we observed. In our results, *P. nunn* coiled massively around the hyphae of both isolates of *P. cinnamomi* and one isolate of *P. parasitica* and subsequently lysed the parasitized hyphae. This phenomenon belongs to the "quick reaction" category in their report, which was applicable to *Pythium ultimum* and *P. vexans* (15). The difference between our results and theirs probably resulted from the use of different isolates of *Phytophthora* in the two studies. Different isolates of the same species of *Phytophthora* (T131 and T593 of *P. parasitica*) used in our study exhibited different reactions to *P. nunn*.

Ours is the first report of the interactions between *P. nunn* and the spores of *Phytophthora* spp. The reproductive propagules of the three *Phytophthora* spp. studied reacted differently to *P. nunn*. The sporangia of *P. citrophthora* and *P. parasitica* were susceptible to, and were coiled and penetrated by, hyphae of *P. nunn;* this was not the case for sporangia of *P. cinnamomi*. The

TABLE 3. Top and root weights of azaleas in the presence of *Pythium nunn*, two *Phytophthora* spp., and three inoculum densities of *Phytophthora* at 8 wk[v]

	Top weight (mg)	Root weight (mg)
Pythium nunn (ppg)[w]		
0	310.7	29.0
300	307.1	32.7
Phytophthora spp.[x]		
P. cinnamomi	284.6	28.1
P. parasitica	333.2	33.6
Phytophthora inoculum density (ppg)[y]		
0	393.5 a[z]	37.6 a
30	268.2 b	29.4 ab
300	265.0 b	25.6 b

[v] See Table 2 for analysis of variance.
[w] *Pythium nunn* was used at initial inoculum densities of 0 and 300 propagules per gram (ppg). Each top and root weight value is a mean of three inoculum densities and two *Phytophthora* spp. with five replicate plants each.
[x] Each top and root weight value is a mean of two *Pythium nunn* treatments and three inoculum densities of *Phytophthora* with five replicate plants each.
[y] Each top and root weight value is a mean of two *Pythium nunn* treatments and two *Phytophthora* species with five replicate plants each.
[z] Means followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's LSD test.

TABLE 4. Analysis of variance for the effects of *Pythium nunn* (Pn) at 300 propagules per gram and three inoculum densities of *Phytophthora parasitica* (ID) on new shoot growth and number of white root tips of sweet orange seedlings[z]

Source of variation	df	Mean square	*F*	*P*
New shoot growth				
Pn	1	700.83	8.25	0.008
ID	2	1,429.03	16.81	0.001
Pn × ID	2	65.03	0.77	1.000
Error	24	85.00		
Number of white root tips				
Pn	1	43.20	0.31	1.000
ID	2	1,599.60	11.56	0.001
Pn × ID	2	120.40	0.87	1.000
Error	24	138.40		

[z] See Table 5 for treatments.

TABLE 5. New shoot growth and number of white root tips of sweet orange seedlings in the presence of *Pythium nunn* at 300 propagules per gram and three inoculum densities of *Phytophthora parasitica* at 8 wk[v]

	New shoot growth (mm)	White root tips (number)
Pythium nunn (ppg)[w]		
0	33.2 a[y]	33.4
300	23.5 b	31.0
Phytophthora parasitica inoculum density (ppg)[x]		
0	40.5 a[z]	46.2 a
50	28.0 b	28.8 b
200	16.6 c	21.6 b

[v] See Table 4 for analysis of variance.
[w] *Pythium nunn* was used at initial inoculum densities of 0 and 300 propagules per gram (ppg). Each shoot growth and root tips value is a mean of three inoculum densities of *Phytophthora parasitica* with five replicate plants each.
[x] Each shoot growth and root tips value is a mean of two *Pythium nunn* treatments with five replicate plants each.
[y] Means followed by the same letter are not significantly different at $P \leq 0.05$ according to Student's *t* test.
[z] Means followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's LSD test.

TABLE 6. Analysis of variance for the effects of *Pythium nunn* (Pn) at 1,000 propagules per gram and three inoculum densities of *Phytophthora parasitica* (ID) on new shoot growth and number of white root tips of sweet orange seedlings[y]

Source of variation	df	Mean square	*F*	*P*
New shoot growth				
Pn	1	30.00	0.05	1.000
ID	2	22,722.03	35.12	0.001
Pn × ID	2	6,048.10	9.35	0.001
Error	24	646.93		
Pn vs. no Pn[z]				
ID = 0	1	7,290.00	11.27	0.002
ID = 30	1	1,188.10	1.84	0.187
ID = 300	1	3,648.10	5.64	0.025
Number of white root tips				
Pn	1	1,428.30	1.58	0.220
ID	2	11,286.23	12.51	0.001
Pn × ID	2	2,727.10	3.02	0.067
Error	24	902.35		
Pn vs. no Pn				
ID = 0	1	160.00	0.18	1.000
ID = 30	1	14.40	0.02	1.000
ID = 300	1	6,708.10	7.43	0.011

[y] See Table 7 for treatments.
[z] Single degree of freedom contrasts for the effect of Pn vs. no Pn at each *Phytophthora* inoculum density.

chlamydospores, antheridia, and oogonia of *P. parasitica* were also more susceptible to *P. nunn* than were those of *P. cinnamomi*. The difference in susceptibility among the various *Phytophthora* spp. to parasitism by *P. nunn* is somewhat anticipated, as species of the same genus often exhibit different reactions to toxicants as well as to many external environmental factors.

Pythium nunn reduced mycelial growth of *P. cinnamomi* and *P. parasitica* on V8C agar after *P. nunn* had been added directly to the mycelia of *Phytophthora* spp. The reason for this might be that the host hyphae stopped growth after they were in contact with *P. nunn* in the "tip-to-host side" interaction reported by Laing and Deacon (14). They reported on the mycoparasitism of *P. nunn* to nine fungal pathogens and described "tip-to-host side" and "side-to-host tip" interactions, both of which could stop the growth of host hyphae. They also reported that growth stoppage was accompanied by host lysis or penetration, which we also observed in our study.

TABLE 7. New shoot growth and number of white root tips of sweet orange seedlings in the presence of *Pythium nunn* at 1,000 propagules per gram and three inoculum densities of *Phytophthora parasitica* at 8 wk[v]

	Pythium nunn inoculum density (ppg)[w]				
	New shoot growth (mm)		White root tips (number)		
	0	1,000	0	1,000	Mean
Phytophthora parasitica inoculum density (ppg)					
0	134.2 a[x,y]	80.2 b	85.2	77.2	81.2 a[z]
30	83.8 a	105.6 a	87.8	85.4	86.6 a
300	0.0 b	38.2 a	0.0	51.8	29.5 b
Mean			57.7	71.5	

[v] See Table 6 for analysis of variance.
[w] *Pythium nunn* was used at initial inoculum densities of 0 and 1,000 propagules per gram (ppg).
[x] Each individual shoot growth and root tips value is mean of five replicate plants. All five plants in the *P. nunn* (0 ppg) + *P. parasitica* (300 ppg) treatment had died within 6 wk.
[y] Means followed by the same letter within each row are not significantly different at $P \leq 0.05$ according to single degree of freedom contrasts.
[z] Means followed by the same letter within each column are not significantly different at $P \leq 0.05$ according to Fisher's LSD test.

The results of our experiments showed that *P. nunn* populations decreased in the nonamended mix, but increased after 1% ground rolled oats were added. This indicated that nutrients were important for population increase in the mix. These results support the report of Paulitz and Baker (21) that *P. nunn* did not increase saprophytically in raw soil unless an organic substrate was added.

Population densities of *P. nunn* were affected by *P. parasitica* in the mix and were less when *Phytophthora* was present than when it was absent. This indicated that *P. nunn* did not consume *Phytophthora* as nutrients as the result of mycoparasitism, but instead the two organisms might have competed for the available nutrients. Paulitz and Baker (21,22) also reported that the suppression of *P. ultimum* by *P. nunn* was due to nutrient competition.

Pythium nunn reduced the population densities of *P. cinnamomi*, *P. citrophthora,* and *P. parasitica* (isolate T593) in oat-amended mix in two of our three experiments. Our data supported the previous work by Paulitz and Baker (21,22) that soil amendment with organic substrates was important in the suppression of pathogen populations by *P. nunn*, and that *P. nunn* was not effective in pathogen suppression in soil without an organic food base such as ground oats. However, population densities of isolate T131 of *P. parasitica* were not reduced by *P. nunn* in each of the two other experiments even in the oat-amended mix. The reasons for the anomalies are unclear. *Phytophthora* spp. are generally known to be poor competitors (18); lower *Phytophthora* populations in the presence of *P. nunn* in the amended mix are to be expected.

Pythium nunn at an initial inoculum density of 300 ppg did not suppress azalea root rot caused by either *P. cinnamomi* or *P. parasitica*, although population densities of both species of *Phytophthora* (at 300 ppg) were reduced by *P. nunn*. The reason for this could be that, in spite of reduced densities, the populations of *Phytophthora* in the mix were still high enough to induce significant disease, which *P. nunn* could not adequately suppress. The poor performance of *P. nunn* as a biocontrol agent against *Phytophthora* may be related to one of the phenomena reported by Laing and Deacon (14). They showed that *P. nunn* was a relatively slow-acting mycoparasite compared with two other known mycoparasitic *Pythium* species (14). Mycoparasitism alone

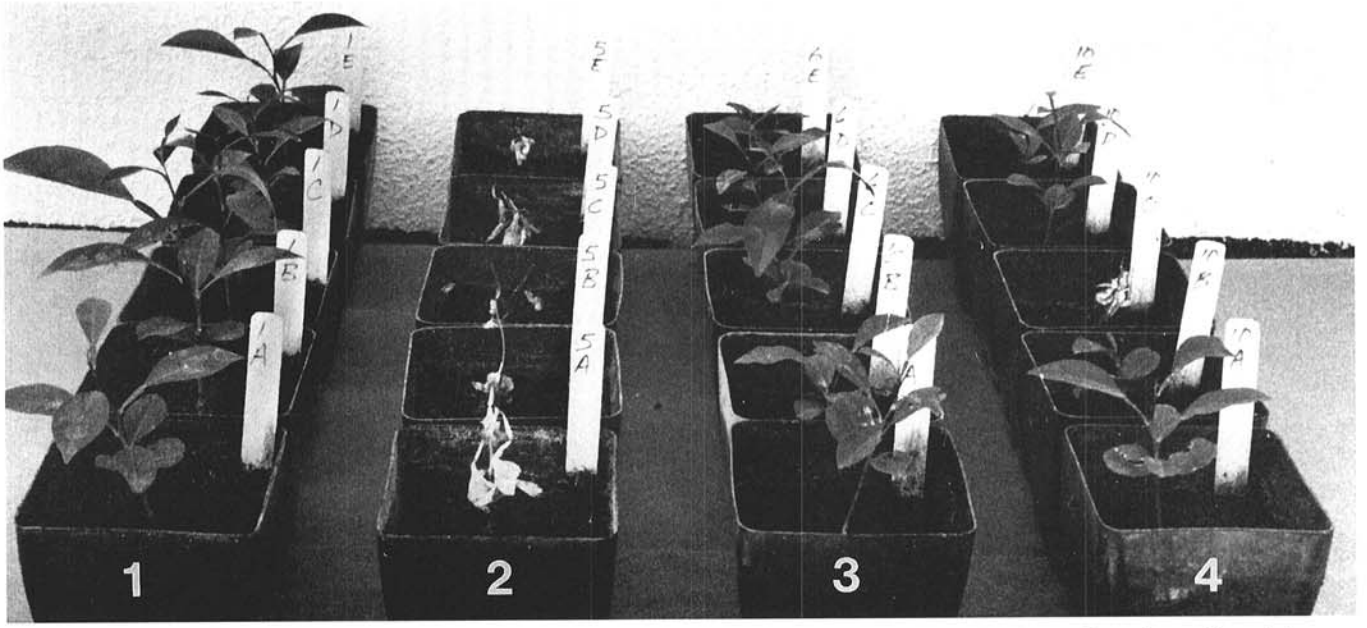

Fig. 6. Suppression of sweet orange root rot, caused by *Phytophthora parasitica,* by *Pythium nunn* at 1,000 ppg. Row 1, no *Phytophthora,* no *P. nunn*; row 2, *Phytophthora* alone; row 3, *P. nunn* alone; row 4, *P. nunn* plus *Phytophthora.* The photograph was taken at 8 wk. All five plants died in the *Phytophthora*-alone treatment (2). Only one of the five plants was dead in the *P. nunn* + *Phytophthora* treatment (4). Growth of sweet orange seedlings in the *P. nunn*-alone treatment (3) was reduced to 60% of that of the control (1), but the roots of these plants appeared healthy.

by *P. nunn*, therefore, was inadequate to accomplish disease suppression in our azalea tests. In the case of suppression of sweet orange root rot caused by *P. parasitica*, *P. nunn* was also not effective at 300 ppg, but significantly suppressed the disease at 1,000 ppg. It appears that *P. nunn* is not as efficient a biocontrol agent in suppressing Phytophthora root rots of woody perennials as in controlling seedling damping-off caused by *Pythium ultimum*.

Pythium nunn at 300 ppg did not exert a deleterious effect on azalea; similar results have been found on other plants by Lifshitz et al (16). They reported its nonpathogenic nature on 11 herbaceous hosts when *P. nunn* was used at a low inoculum density (about 100 ppg). Paulitz and Baker (21,22) also found *P. nunn* to exert no harmful effects on cucumber seedlings at 300 ppg, an inoculum density that was effective in suppressing *P. ultimum* on cucumber in numerous tests. Prior to our study, however, no initial inoculum densities greater than 300 ppg had been reported on *P. nunn* for its effect on plants. In our work, *P. nunn* was tested at the initial inoculum densities of 300 and 1,000 ppg, the latter being admittedly an unusually high concentration. While root rot suppression was achieved by *P. nunn* at 1,000 ppg, this high inoculum density also slightly reduced the top growth of sweet orange seedlings. The roots of these affected seedlings, however, appeared healthy, with no significant reduction in the number of white root tips, and no parasitism on sweet orange roots was demonstrated in our work.

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