Evaluation of *Phytophthora parasitica* var. *nicotianae* for Biocontrol of *Phytophthora parasitica* on *Catharanthus roseus*

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ABSTRACT

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Isolates of *Phytophthora parasitica* var. *nicotianae* were selected for biocontrol of *Phytophthora parasitica*, which causes preemergence damping-off of *Catharanthus roseus*. Isolates of *P. p. nicotianae* pathogenic to tobacco were weakly pathogenic on *C. roseus* and variable in their biocontrol of damping-off. Rice grain cultures of *P. parasitica* (14–21 days old) at 0.05 g per 25.4 cm^2 plug tray were optimal for screening potential biocontrol isolates in the greenhouse. After screening 41 isolates in preliminary experiments, 11 isolates were tested in three repeated experiments. Three isolates of *P. p. nicotianae* (402, 602, and 723) that represented the range of effective control of *P. parasitica* were chosen for further study. The isolates selected as potential biocontrol agents, however, stunted ($P = 0.05$) main-root extension in *C. roseus*. Above-ground growth of *C. roseus* also was stunted initially by *P. p. nicotianae*, as first and second leaves were shorter ($P = 0.05$) on plants grown with each isolate compared to leaves of plants in the uninfested control. Flowering was not affected. *P. p. nicotianae* was recovered from roots, crowns, and stems of *C. roseus* seedlings, but not to the same extent as was *P. parasitica*. Severity of preemergence damping-off caused by isolates of *P. p. nicotianae* was related directly to the amount of plant tissue colonized by each isolate. Isolate 723 caused the least amount of stunting, colonized the least amount of host tissue, and was the least pathogenic on *C. roseus* while giving 52% control of isolate 336 of *P. parasitica*. In addition, isolate 723 was effective (range 13–73%) in protecting *C. roseus* from several additional isolates of *P. parasitica*. Metalaxyl-insensitive isolates of *P. parasitica* were used in population studies with isolate 723 in peat–vermiculite medium seeded to *C. roseus*. Incorporation of metalaxyl into one-half of the assay plates permitted enumeration of both antagonist and pathogen in the growth medium. Populations of *P. parasitica* were lowered when *P. p. nicotianae* was present. Apparently, one possible mechanism of biocontrol is direct suppression of the pathogen population through competition for nutrients. However, use of *P. p. nicotianae* for biocontrol of preemergence damping-off of *C. roseus* caused by *P. parasitica* does not seem promising unless more effective nonpathogenic isolates can be found.

Catharanthus roseus (L.) G. Don is a popular annual bedding plant in the southern half of the United States because of its heat, drought, salt, and pollution tolerance (11,19). *C. roseus* is usually propagated from seeds in commercial greenhouses where *Phytophthora parasitica* Dastur can cause preemergence and postemergence damping-off. *P. parasitica* is also a destructive pathogen that causes damping-off, crown rot, leaf blight, and fruit rot on many greenhouse plants, including African violet, gloxinia, petunia, chrysanthemum, and viola (1,7, 10). *P. parasitica* was first reported on *C. roseus* in India by Dastur in 1916 (4), and in the United States in California in 1977 (9,13). Several fungicides are effective for control of *P. parasitica* on *C. roseus*, e.g., soil drenches of fosetyl Al (K. A. Holmes and D. M. Benson, *unpublished*), metalaxyl, and a formulation of benomyl and metalaxyl (20). Metalaxyl-insensitive isolates have been found in California, however, which may limit the value of fungicides in the future (8).

Biological control of *P. parasitica* may offer an alternative to dependence on fungicides. Nonpathogenic or weakly pathogenic organisms or a related species of the pathogenic organism have been used in biological control (2). Nonpathogenic species may be effective as biocontrol agents through a variety of mechanisms, which include competition for infection sites or nutrients, mycoparasitism, antibiosis, or induced resistance (2). Nonpathogenic or weakly pathogenic *Phytophthora* species, including *P. infestans, P. cactorum,* and *P. parasitica,* have been reported to protect potato tubers from *P. infestans* (15), soybean seedlings from *P. megasperma* var. *sojae* (16), and avocado seedlings from either *P. cinnamomi* or *P. citricola* (5), respectively.

The objectives of this research were to evaluate isolates of *P. parasitica* var. *nicotianae* from tobacco as potential biocontrol agents for preemergence damping-off of *C. roseus* caused by *P. parasitica,* establish criteria for selecting the best antagonistic isolates, and follow populations of *P. parasitica* in the presence of *P. p. nicotianae*.

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MATERIALS AND METHODS

Inoculum age and concentration of *P. parasitica*. Three 7-mm-diameter disks of mycelium of *P. parasitica* isolate 336 from CMA were placed in flasks containing 25 g of rice and 18 ml of deionized water that had been autoclaved. Every 7 days for 21 days, additional flasks of rice grains were seeded with isolate 336 so that pathogen inoculum 7, 14, and 21 days old was available at seeding. The colonized rice was pulverized, passed through a 2-mm sieve, and diluted with sand. Inoculum concentrations of 0.05, 0.085, and 0.15 g per 81-cell, 25.4-cm^2 plug tray were chosen based on preliminary experiments. Inoculum was incorporated into a 150-cm^3 reserved portion of a peat–vermiculite medium and sprinkled onto the surface of trays seeded with *C. roseus* as described below. Stand counts were taken every 3 days and converted to percent preemergence damping-off based on stand count for isolate 336 of *P. parasitica* compared to the uninfested control. The experiment was conducted three times. Data were analyzed with the PROC GLM procedure of PC SAS (SAS Institute, Cary, NC), and means were separated by the Waller-Duncan *k*-ratio *t* test.

Screening isolates for control. Isolates of *P. p. nicotianae* causing black shank in flue-cured tobacco were obtained from H. D. Shew, Department of Plant Pathology, North Carolina State University, Raleigh. In preliminary experiments, isolates of *P. p. nicotianae* caused some stunting on transplanted seedlings of *C. roseus,* but no leaf chlorosis or root necrosis was observed. A pathogenic isolate of *P. parasitica* (336) was isolated from *C. roseus*. Isolates were grown on PPP agar (6) and then transferred to cornmeal agar (CMA). Three 7-mm-diameter disks of mycelium from CMA were placed in a 250-ml flask of rice grains (25 g rice, 18 ml deionized water) that had been autoclaved twice for 30 min. Fungi were allowed to colonize the rice for about 14 days at room temperature. Colonized rice grains then were pulverized without additional water in a blender and sieved through a 9-mesh screen (2-mm openings) to select particles that could be distributed in small quantities.

A mixture of peat moss (sieved through a screen with 1.2-cm openings) and vermiculite (1:1 [v/v]) plus lime at 5.9 g/1,000 cm^3 of medium was moist-

ened to bring the water content to about 60% by weight. The medium was then divided into 6,400-cm^3 aliquots and placed in clear plastic bags. Each aliquot of medium was amended by thorough mixing with 19.2 g of the pulverized rice particles colonized by hyphae of a selected isolate of *P. p. nicotianae*. The amended medium was incubated under constant light for a week at room temperature. After incubation, all but 600 cm^3 of the amended medium was put into four 81-cell plug trays (25.4 × 25.4 × 2.5 cm) and seeded with *C. roseus*. A 150-cm^3 portion of the reserved medium from the 6,400-cm^3 aliquot was infested with 0.05 g of pulverized rice colonized with *P. parasitica* and sprinkled over the seed in each tray to simulate surface contamination of the soilless medium by the pathogen. An infested control (336), uninfested control, and fungicide control (fosetyl Al at 1.2 g a.i./L applied at 12 ml per plug tray) were included, with most groups of isolates of *P. p. nicotianae* tested. An uncolonized rice control was included once to check for phytotoxic effects on seed germination. The plug trays were placed under a mist system that cycled on for 2 min twice a day. Stand counts were taken on day 7, 10, 13, 16, 21, and 28. Preemergence damping-off was calculated based on stand counts of the uninfested control. Over all preliminary trials, 41 isolates of *P. p. nicotianae* were screened, and the 11 that gave the greatest protection from preemergence damping-off were selected for additional experiments. Data were analyzed with the PROC GLM procedure of PC SAS (SAS Institute, Cary, NC), and means were separated by the Waller-Duncan *k*-ratio *t* test. Since variances from three runs of the experiment with the 11 isolates were homogenous, data were pooled for analysis and presentation.

Effect of potential antagonists of *P. p. nicotianae* on *C. roseus*. Damping-off of *C. roseus* induced by isolates of *P. p. nicotianae* was tested in the absence of *P. parasitica*. The medium was amended at the rate of 19.2 g of pulverized rice particles colonized by a given isolate of *P. p. nicotianae* per 6,400 cm^3 peat–vermiculite. A 7-day incubation period in plastic bags was used before trays were filled and seeded. Seeds were covered with a reserved portion of the incubated medium as described above. Trays were placed under the mist system, and stand counts were made as seedlings emerged.

To determine if isolates of *P. p. nicotianae* had an effect on root growth of seedlings, *C. roseus* was seeded in peat–vermiculite medium amended with isolate 402, 602, or 723 of *P. p. nicotianae,* in an uninfested control, or in medium with isolate 336 of *P. parasitica* added as a topdressing. After seedling emergence, five seedlings from each replication were selected randomly every 3 days and removed along with the entire plug. Most of the medium adhering to each seedling was rinsed off in distilled water, and the seedlings were placed on paper towels to absorb excess water. The length of the taproot on each seedling was used as the measure of root growth.

The region of colonization on seedlings of *C. roseus* by isolates of *P. p. nicotianae* was tested. Isolates 402, 602, and 723 of *P. p. nicotianae* and isolate 336 of *P. parasitica* were grown on rice grains for 21 days. Isolates of *P. p. nicotianae* but not of *P. parasitica* were incubated in the peat–vermiculite medium for 7 days prior to seeding as described above. As a control, 0.05 g per tray of pulverized rice grains colonized with *P. parasitica* were sprinkled over seeds in one set of plug trays without *P. p. nicotianae*. There were four replications for each isolate arranged in a randomized complete-block design on the mist bench. Plug trays were misted for 2 min three times each day. Five seedlings from each treatment were sampled at random at 6, 9, 12, and 15 days after seeding by removing the entire plug. The medium was shaken from the roots, and the roots were soaked in sterile deionized water to remove any remaining medium. The seedling and roots were placed in 0.5% sodium hypochlorite for 30 sec and then rinsed in sterilized deionized water and placed on paper towels to absorb excess water. Seedlings and roots were placed on PARP (12) selective agar as modified by Shew (18). After about 48 hr, any *Phytophthora* colonies present were counted and the regions of origin were recorded. The regions were stem and leaves, crown, middle section of the root, and root tip. Percent recovery of *Phytophthora* spp., by region, was calculated based on cumulative data collected over the sampling period. Stand counts were taken prior to seedling removal to determine the amount of preemergence damping-off that resulted in each treatment.

To determine if isolates of *P. p. nicotianae* had an effect on the rate of leaf expansion, *C. roseus* was seeded in a medium amended with isolate 402, 602, or 723 of *P. p. nicotianae,* in an uninfested control, or in a control with *P. parasitica* only. Observations were made as first and second leaves expanded from small buds to leaves about 2 mm long. Measurements of the length of the first leaves were taken on day 20 and day 28, and second leaves were measured on day 28. Eight leaves from different plants for each of four replications per treatment were selected at random for measurement. Twenty-eight days after seeding, two seedlings per replication were transferred to 10-cm-diameter pots and grown to flowering. Seedlings from uninfested trays were included as a control.

***P. p. nicotianae* as a biocontrol of other pathogenic isolates of *P. parasitica*.** Isolates 736 and 337 of *P. parasitica* from *C. roseus* were obtained from plant samples submitted to the Plant Disease and Insect Clinic of North Carolina State University to compare the efficacy of isolate 723 of *P. p. nicotianae* to protect *C. roseus* from other isolates of the pathogen. Isolate 723 was amended as pulverized rice grains to the peat–vermiculite medium 7 days before seeding (incubation in plastic bags as above) while pathogenic isolates 736 or 337 of *P. parasitica* were applied as colonized rice particles at the rate of 0.05 g/150 cm^3 of medium over the seeds. Stand counts were taken, and percent preemergence damping-off was determined. Infested controls without *P. p. nicotianae*

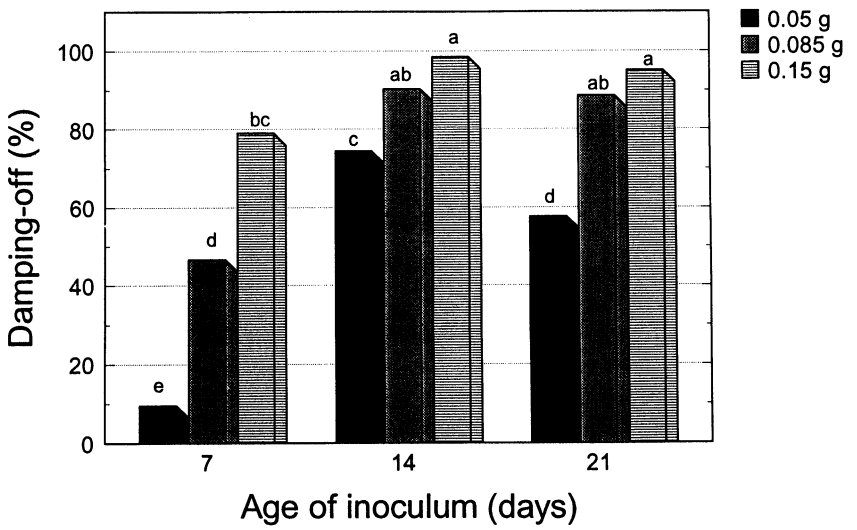

Fig. 1. Effects of the inoculum concentration expressed as grams of pulverized, colonized rice particles added per 25.4 cm^2 of medium, and inoculum age on the amount of preemergence damping-off on *Catharanthus roseus* caused by *Phytophthora parasitica* in plug trays under an overhead mist system.

were included with each isolate of *P. parasitica.*

Population studies with metalaxyl-insensitive isolates of *P. parasitica.* Population density of isolate 723 of *P. p. nicotianae* alone and in combination with either pathogenic, metalaxyl-insensitive isolate 015F or 014F of *P. parasitica* isolated from *C. roseus* and supplied by D. M. Ferrin, Department of Plant Pathology, University of California, Riverside, was determined. Isolate 723 was added at 21 g of pulverized rice particles colonized with *P. p. nicotianae* per 7,000 cm^3 of medium (equals 3.9 g/1,300 cm^3 plug tray) and incubated in plastic bags for 7 days as described above. Isolates of *P. parasitica* were added after seeding at the rate of 0.05 g of colonized rice particles per plug tray as described above. Controls were included with isolates 336, 014F, or 015F of *P. parasitica* alone. Trays were sampled at seeding and every 3 days for 21 days. One plug from each of four replications per treatment was sampled at random. Bulked plugs containing roots and medium on a treatment basis were mixed in a plastic bag. For each treatment, two 5-g samples were taken from the plastic bag, diluted in sterile water, and spread on 20 plates each of PARP agar or PARP agar amended with 100 ppm metalaxyl. Three additional subsamples from each treatment were oven-dried for medium moisture determination. Population densities were determined as colony-forming units (cfu) per gram dry weight of medium. Population densities of metalaxyl-insensitive isolates 014F and 015F of *P. parasitica* were estimated on PARP agar amended with metalaxyl. Populations of metalaxyl-sensitive isolate 723 of *P. p. nicotianae* were estimated by subtraction of colony-forming units on PARP with metalaxyl from those on PARP without metalaxyl. Stand counts were taken, and percent preemergence damping-off was determined. The experiment was repeated with similar results.

RESULTS

Inoculum age and concentration. Preemergence damping-off of *C. roseus* increased with inoculum age and concentration of *P. parasitica.* For a given inoculum age class (7, 14, or 21 days old), increasing inoculum concentration resulted in increased preemergence damping-off (Fig. 1). However, percent damping-off for each concentration of 21-day-old inoculum was similar to the percent at the same concentration of 14-day-old inoculum. The 7-day-old inoculum caused less damping-off at each concentration.

Screening *P. p. nicotianae* isolates for biocontrol. Isolates of *P. p. nicotianae* were variable in protection of *C. roseus* from preemergence damping-off caused by *P. parasitica.* From an initial pool of 41 isolates of *P. p. nicotianae* tested in preliminary experiments, 11 were chosen for further evaluation. The percent preemergence damping-off that resulted when *P. p. nicotianae* isolates were used to protect *C. roseus* from *P. parasitica* ranged from a low of 29% with isolate 602 to a high of 65% with isolate 314 (Fig. 2). Damping-off in the infested control (isolate 336 of *P. parasitica*) averaged 76% over the three experiments. No damping-off was observed in the uninfested control. Damping-off of *C. roseus* was less ($P = 0.05$) than in the infested control when isolates 602, 723, 256, 404, 519, 85, 402, or 610 of *P. p. nicotianae* were used (Fig. 2). Damping-off in media drenched with the fungicide fosetyl Al was not significantly different than damping-off in the infested control. Emergence of *C. roseus* in the uncolonized rice control was not significantly different ($P = 0.05$) from that in the uninfested control. Three isolates were selected for further evaluation: 602 and 723, which resulted in the best protection, and 402, which was intermediate.

Effect of selected isolates of *P. p. nicotianae* on damping-off, root development, and colonization of *C. roseus.* In growth medium uninfested with *P. parasitica,* isolates 402 and 602 of *P. p.*

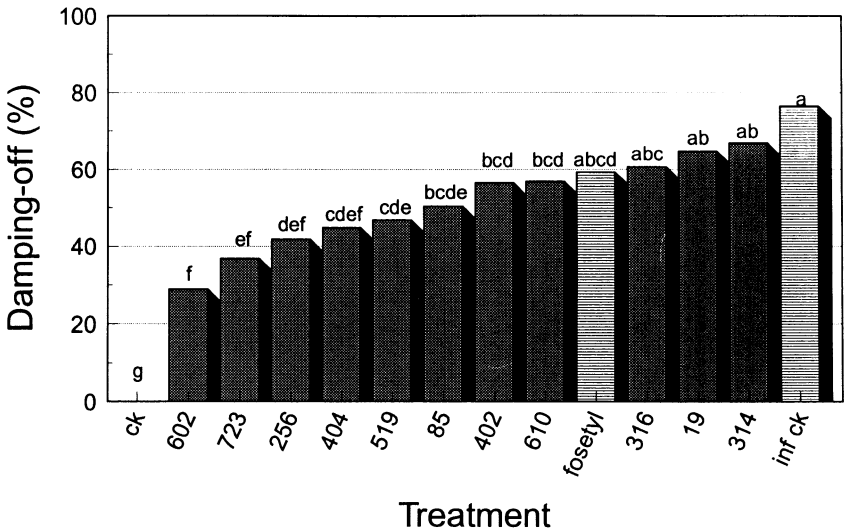

Fig. 2. Effect of 11 selected isolates of *Phytophthora parasitica* var. *nicotianae* on preemergence damping-off caused by *Phytophthora parasitica* on *Catharanthus roseus* in plug trays. Isolate 336 of *P. parasitica* in the absence of an isolate of *P. p. nicotianae* served as the infested control. Fosetyl Al was applied to plug trays infested with *P. parasitica* as a fungicide control. Results of three experiments were pooled since the variances were homogenous. Bars capped with the same letter are not significantly different according to the Waller-Duncan *k*-ratio *t* test, $k = 100$, $P = 0.05$.

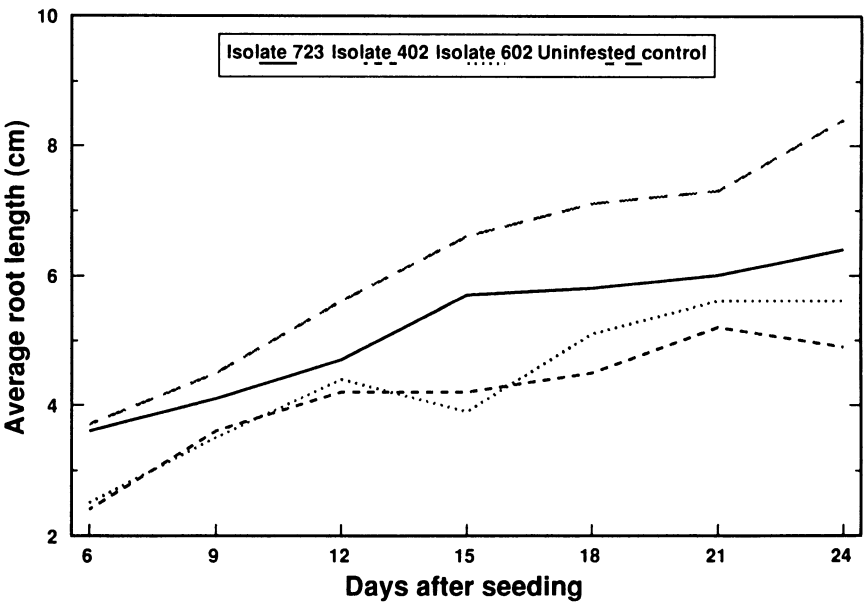

Fig. 3. Effect of isolates 402, 602, and 723 of *Phytophthora parasitica* var. *nicotianae* on main root length of *Catharanthus roseus* in plug trays under an overhead mist system in a greenhouse. Root lengths at day 24 marked with the same letter are not significantly different according to the Waller-Duncan *k*-ratio *t* test, $k = 100$, $P = 0.05$.

nicotianae induced 15 and 8% preemergence damping-off, respectively, while isolate 723 induced 2%. Over several experiments, the low rate of preemergence damping-off induced by isolate 723 was not significantly different ($P = 0.05$) from the uninfested control where no damping-off was observed.

Root development of *C. roseus* as measured by root length was less ($P = 0.05$) than in the uninfested control as early as 6 days after seeding for isolates 402 and 602 in the absence of *P. parasitica* (Fig. 3). However, for isolate 723, root development was not restricted ($P = 0.05$) until day 12 compared to the uninfested control, as determined by univariate analysis of variance at each sample date. At 24 days, root development was less ($P = 0.05$) for seedlings in medium amended with isolates 402, 602, and 723 of *P. p. nicotianae* compared to the uninfested control.

Colonization of seedlings by the isolates of *P. p. nicotianae* increased from 0 to 10% at 6 days, depending on isolate, up to 35% colonization at day 12 for isolate 402 (Fig. 4). Even though seedling colonization in the control (isolate 336, *P. parasitica*) continued to increase after 9 days, colonization of seedlings by isolates of *P. p. nicotianae* was static after 9 days. Rates of seedling colonization among isolates 402, 602, and 723 were not significantly different ($P = 0.05$) over time. Seedling colonization by isolate 336 reached 90% by day 12 on seedlings that emerged.

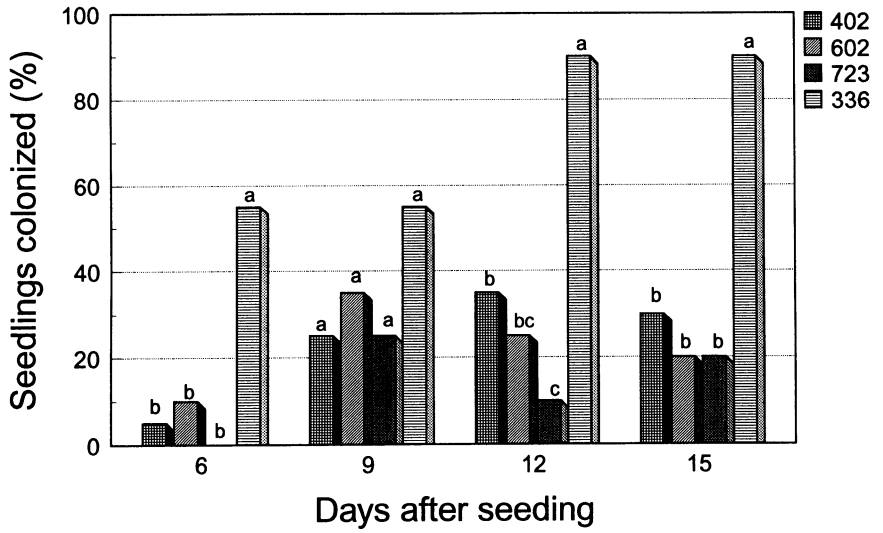

Fig. 4. Colonization of seedlings of *Catharanthus roseus* by isolates 402, 602, and 723 of *Phytophthora parasitica* var. *nicotianae* and by isolate 336 of *Phytophthora parasitica* between 6 and 15 days after seeding in plug trays in a greenhouse. Values represent the mean of four replications per sampling date. Bars capped with the same letter within sampling dates are not significantly different according to the Waller-Duncan *k*-ratio *t* test, $k = 100$, $P = 0.05$.

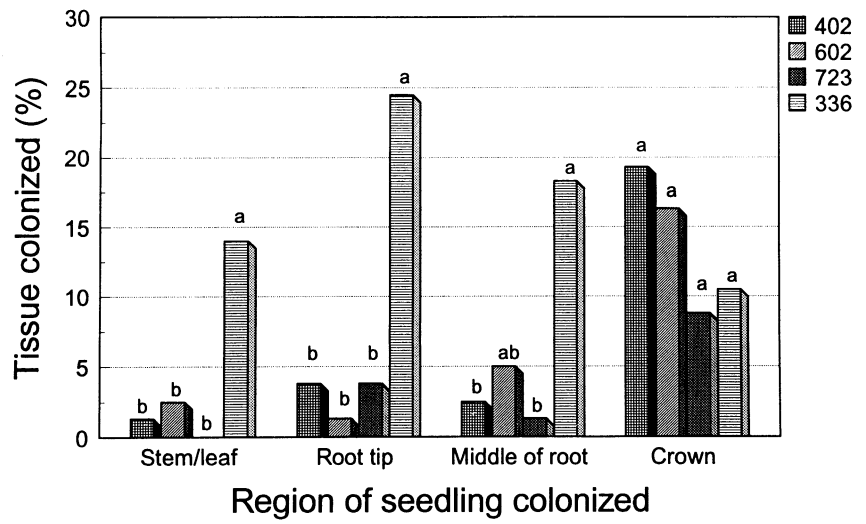

Fig. 5. Percent of tissue colonized by region for *Phytophthora* spp. from *Catharanthus roseus* seedlings in plug trays under an overhead mist system in a greenhouse. Values represent the mean of four replications per sampling date. Bars capped with the same letter for each tissue region are not significantly different according to the Waller-Duncan *k*-ratio *t* test, $k = 100$, $P = 0.05$.

Isolates of *P. p. nicotianae* colonized the crown region of seedlings to the greatest extent (9–19%) over the four sampling dates, even though seedlings appeared symptomless at sampling (Fig. 5). No significant difference ($P = 0.05$) in the extent of crown colonization was found among isolates 402, 602, and 723, and isolate 336 of *P. parasitica.* Colonization of other regions was less than 5% for isolates of *P. p. nicotianae* but 13–24% for *P. parasitica,* depending on the region. Unlike the colonization pattern for isolates of *P. p. nicotianae, P. parasitica* colonized other regions more extensively than the crown (Fig. 5). Although isolate 723 colonized crown and below-ground tissues, this isolate was not recovered from above-ground stem and leaf tissue.

Root length was not correlated with seedling colonization. The average root length of seedlings colonized by *P. p. nicotianae* was about 3.9 cm, the same as the average of seedlings not colonized but grown in media amended with *P. p. nicotianae.* For seedlings sampled from a medium infested with *P. parasitica,* the root length was 4.6 cm for seedlings colonized compared to 4.5 cm for seedlings not colonized.

The first leaves of seedlings grown in media amended with isolates of *P. p. nicotianae* took 4 days longer to begin expanding than did the first leaves on seedlings in the unamended medium. After 28 days, the rate of first- and second-leaf expansion was slower ($P = 0.05$) for seedlings in media amended with isolates of *P. p. nicotianae* compared to the uninfested control (Table 1). Not only were leaves on seedlings in the uninfested control larger at each sampling period, they were greener and more vigorous in appearance than those in media amended with isolates of *P. p. nicotianae* or infested with isolate 336 of *P. parasitica.* Representative seedlings from each treatment above were transplanted to 10-cm-diameter pots for further observations of growth, development, and flowering. Over a subsequent 15-day period, damping-off occurred in 25, 12, and 12% of the seedlings in media with isolate 602 of *P. p. nicotianae,* the fungicide control, and the infested control (*P. parasitica*), respectively; whereas no disease was observed on seedlings transplanted from media amended with isolates 402 and 723 of *P. p. nicotianae* or the uninfested control. Plants in all treatments started flowering during the same 10-day period.

Efficacy of *P. p. nicotianae* for biocontrol of other isolates of *P. parasitica.* Biological control of Phytophthora damping-off caused by isolates 337 and 736 (from *C. roseus*) and the metalaxyl-insensitive isolates 014F and 015F of *P. parasitica* was evaluated with isolate 723 of *P. p. nicotianae* under standard conditions as described above. Isolates 337 and

736 caused preemergence damping-off of *C. roseus,* but isolate 337 was not as virulent on *C. roseus* as were isolates 336 and 736. Preemergence damping-off caused by isolate 337 was only 29–34% and not significantly different ($P = 0.05$) from that caused by isolate 337 in the presence of isolate 723 of *P. p. nicotianae* (Table 2). However, preemergence damping-off caused by isolates 736 (53–88%) and 336 (73–83%) of *P. parasitica* was less (15–45%) in the presence of isolate 723.

Isolate 723 of *P. p. nicotianae* was inoculated to tobacco and reisolated in an attempt to improve biocontrol activity towards *P. parasitica.* The isolate designated 723T was no more effective in protection of *C. roseus* than the original isolate (Table 2).

The metalaxyl-insensitive isolates 014F and 015F of *P. parasitica* were pathogenic on *C. roseus,* causing 55 and 68% preemergence damping-off, respectively, compared to 85% induced by isolate 336. *P. p. nicotianae* isolate 723 was also effective against the metalaxyl-insensitive isolates of *P. parasitica.* In the presence of isolate 723, preemergence damping-off was 33 and 38% with isolates 014F and 015F, respectively, compared to 55 and 68% without isolate 723.

Population studies with metalaxyl-insensitive isolates of *P. parasitica.* Combined populations of *P. p. nicotianae* and either isolate 014F or 015F of *P. parasitica* were similar over the 21-day sampling period with between 9,000 and 20,000 cfu/g dry weight of medium (Figs. 6 and 7). High initial populations of *P. p. nicotianae* at seeding resulted from preincubation of *P. p. nicotianae* in media for 7 days prior to seeding. On the other hand, populations of isolates 014F and 015F increased from near 10 cfu/g at seeding to 2,000–4,000 cfu/g between 6 and 21 days after seeding. In contrast, populations of isolates 014F and 015F in the presence of *P. p. nicotianae* ranged from 10 to 1,000 cfu/g between 6 and 21 days after seeding. Isolates 014F and 015F, in treatments without isolate 723 of *P. p. nicotianae,* had population patterns more similar to that of isolate 336 of *P. parasitica* (*data not shown*), which was not metalaxyl insensitive.

DISCUSSION

Several isolates of *P. p. nicotianae,* including 602, 723, 256, 404, 519, 85, 402, and 610, protected *C. roseus* from preemergence damping-off caused by *P. parasitica.* The amount of protection observed with these isolates ranged from 62% with the best (602) to 26% with the least effective (610 and 402). In other tests, isolate morphology, growth rate, and county of origin in North Carolina had no correlation with protection of *C. roseus* from preemergence damping-off (K. A. Holmes and D. M. Benson, *unpublished*). The extent of protection was dependent on the severity of the disease. When there was very severe disease in the infested control (i.e., 90% damping-off) there was also greater damping-off in treatments with the biocontrol isolates. When there was less disease in the infested control, there was also less in the treatments with isolates 402, 602, and 723 of *P. p. nicotianae.* Variations in severity were probably due to fluctuations in environmental conditions.

The fungicide fosetyl Al failed to protect *C. roseus* from damping-off in these experiments. Since the fungicide was applied as a spray to the surface of the medium in the plug tray after seeding, it is possible that the mist system did not provide a large enough volume of water to move the fungicide into the potting medium. In other experiments where fosetyl Al and metalaxyl have been applied as soil drenches to the medium, excellent control of damping-off was observed (D. M. Benson, *unpublished*).

Preemergence damping-off of *C. roseus* was usually near 70% when inoculum was between 14 and 21 days old at a concentration of 0.05 g of *P. parasitica*-colonized rice particles per tray (25.4 cm^2). However, effects of inoculum age and concentration possibly were sensitive to temperature, soil moisture, or some other factors, because the amount of preemergence damping-off varied among experiments. The most critical time for the development of *P. parasitica* and for protection of *C. roseus* by isolates of *P. p. nicotianae* seemed to be within the first 7 days after seeding. During this time, soil moisture increased from about 69% at seeding to about 82% as the medium was removed from the plastic bags and placed in trays under the mist system. Provided adequate nutrients were available, sporangia would be produced and zoospores released by both pathogen and antagonist, which could produce more disease or give protection (9,17).

Isolates of *P. p. nicotianae* in the medium free of *P. parasitica* were pathogenic to *C. roseus,* although percent damping-off was low for most isolates and not different than the uninfested control, where no damping-off occurred. Pathogenicity of isolates of *P. p. nicotianae* to *C. roseus* is a major concern in implementation of this biocontrol system. Even though virulence is low, concern would be raised in greenhouse production environments where some crop loss could occur by introduction of the biocontrol agent in the absence of *P. parasitica.*

Root development of *C. roseus* was restricted by isolates of *P. p. nicotianae,* but the roots continued to grow. Plants protected with isolates of *P. p. nicotianae* may need to be transplanted later than plants grown in antagonist-free medium, since root and leaf development was delayed.

The first and second leaves of the seedlings grown in medium with isolates of *P. p. nicotianae* expanded at a slower rate during the first 2–3 wk after emergence, but the plants flowered at the same

Table 1. Effect of isolates 402, 602, and 723 of *Phytophthora parasitica* var. *nicotianae* compared to isolate 336 of *Phytophthora parasitica* and a fungicide control (fosetyl Al) on the length of first and second leaves of *Catharanthus roseus* in plug trays under a mist system in the greenhouse 20 and 28 days after seeding

	Leaf measurements (mm)[y]		
	Day 20	Day 28	
Treatment	1st leaf	1st leaf	2nd leaf
602	6 cd	21 b	9 bc
723	6 cd	20 b	6 c
402	5 d	20 b	7 bc
Infested control (336)	11 b	21 b	10 b
Fosetyl Al control (336)[z]	9 bc	19 b	6 c
Uninfested control	14 a	26 a	16 a

[y]Values followed by the same letter within a column are not significantly different at $P = 0.05$ according to Duncan's multiple range test.
[z]Fosetyl Al was applied to plug trays infested with isolate 336 of *P. parasitica* as a fungicide control.

Table 2. Protection of *Catharanthus roseus* from three isolates of *Phytophthora parasitica* (336, 337, and 736) by isolate 723 of *Phytophthora parasitica* var. *nicotianae* in plug trays under a mist system in a greenhouse

	Preemergence damping-off (%)	
Treatment	Run 1	Run 2
Uninfested control	0 e[y]	0 d
723 alone	6 e	11 cd
723T[z] alone	6 e	6 d
723 + 337	26 d	16 bcd
723 + 736	29 d	15 bcd
723 + 336	45 bc	31 b
723T + 336	45 b	14 bcd
337 alone	34 cd	29 bc
736 alone	53 b	88 a
336 alone	73 a	83 a

[y]Values followed by the same letter within a column are not significantly different at $P = 0.05$ according to the Waller-Duncan k-ratio t test, $k = 100$, $P = 0.05$.
[z]723T is isolate 723 reisolated from a tobacco plant.

time as uninfested controls. Dolan et al (5) also observed stunting of avocado plants protected from Phytophthora root rot in soil amended with the weak pathogen *P. parasitica.*

Seedling colonization by *P. p. nicotianae* was not necessarily important for biocontrol effectiveness, since a low percentage of seedling colonization was observed. Instead, colonization of *C. roseus* seedlings by *P. p. nicotianae* was related to the virulence of the isolates.

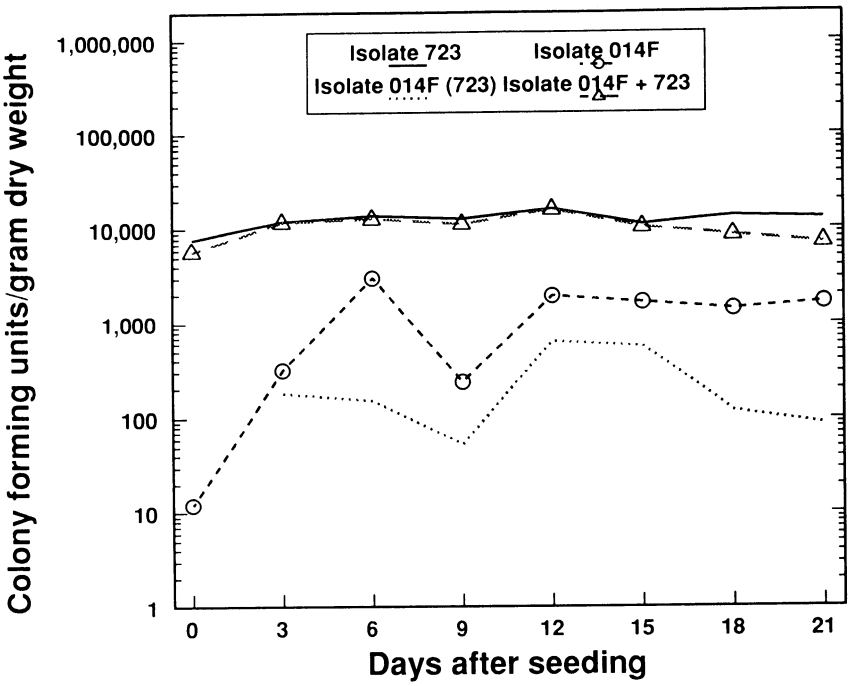

Fig. 6. Population density for *Phytophthora parasitica* var. *nicotianae* (isolate 723) and *Phytophthora parasitica* (isolate 014F), and for the isolates in combination (isolate 014F[723]), as well as the combined *Phytophthora* population (isolates 014F + 723) in plug trays seeded with *Catharanthus roseus* in a peat–vermiculite medium under a mist system in a greenhouse over 21 days. Isolate 723 was incubated in a moistened peat–vermiculite medium in plastic bags on the laboratory bench for 7 days prior to seeding.

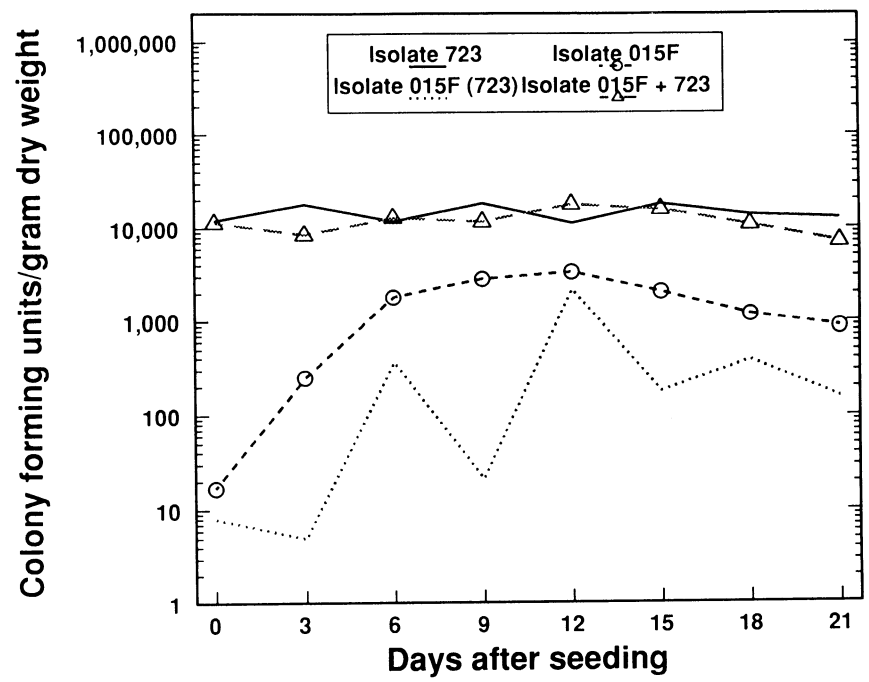

Fig. 7. Population density for *Phytophthora parasitica* var. *nicotianae* (isolate 723) and *Phytophthora parasitica* (isolate 015F), and for the isolates in combination (isolate 015F[723]), as well as the combined *Phytophthora* population (isolates 015F + 723) in plug trays seeded with *Catharanthus roseus* in a peat–vermiculite medium under a mist system in a greenhouse over 21 days. Isolate 723 was incubated in a moistened peat–vermiculite medium in plastic bags on the laboratory bench for 7 days prior to seeding.

Of the three antagonists tested, isolate 402 caused the most preemergence damping-off and colonized the greatest percent of seedlings. As colonization of seedling tissue was not correlated with the stunting of seedling growth, *P. p. nicotianae* could possibly be producing a soluble inhibitory substance. Csinos and Hendrix (3) observed an isolate of *P. cryptogea,* nonparasitic to tobacco, that caused a reduction in the growth of tobacco by producing a toxin.

P. p. nicotianae and *P. parasitica* probably are not competing for the same infection sites on the plant, as the percent recovery of *P. p. nicotianae* was so low that many sites would still be available for *P. parasitica.* Also, in tissue colonization studies, *P. p. nicotianae* was recovered on selective media the most from the crown region of seedlings. In contrast, *P. parasitica* was recovered the most from regions other than the crown.

Isolate 723 gave the best protection of *C. roseus* from preemergence damping-off, stunted the roots the least, and caused the least amount of the disease itself. Isolate 723 protected *C. roseus* from other isolates of *P. parasitica,* including two metalaxyl-insensitive isolates from *C. roseus* in California, as well as from isolate 336 used for the initial screening. This suggested that isolate 723 would probably be able to protect *C. roseus* from a variety of *P. parasitica* isolates. The fact that up to 3.9 g of pulverized rice particles of isolate 723 of *P. p. nicotianae* per 1,300-cm^3 plug tray was necessary for control of *P. parasitica* may mean that competition for available nutrients in the medium occurred. Millard and Taylor (14) suggested that protection of potatoes by *Streptomyces praecox* from *Streptomyces scabies* could be due to *S. praecox* obtaining the food supply before *S. scabies.* In our system, pulverizing colonized rice particles prior to addition of the antagonist to the growth medium could expose numerous new uncolonized surfaces on the rice particles for growth and sporulation by *P. p. nicotianae.* Since *P. p. nicotianae* was added prior to seeding, the large populations that developed prior to seeding could suppress *P. parasitica* through competition for nutrients.

Population studies with metalaxyl-insensitive isolates 014F and 015F of *P. parasitica* and isolate 723 of *P. p. nicotianae* demonstrated that *P. parasitica* did not have a great effect on populations of *P. p. nicotianae.* On the other hand, the populations of both pathogenic isolates were lower in the presence of isolate 723. Along with the suppressive effect on populations of *P. parasitica,* some control of preemergence damping-off was observed. The lower populations of *P. parasitica* could be due to competition with the established population of *P. p. nicotianae* for nutrients. This mechanism of control is most likely, as

P. p. nicotianae suppressed populations of *P. parasitica* without extensive colonization of seedlings.

Several isolates of *P. p. nicotianae* proved effective in the protection of *C. roseus* from *P. parasitica* during seed germination and emergence. However, other effective isolates of *P. p. nicotianae,* which are not pathogenic to *C. roseus,* must be found if this system is to become practical for growers.

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