Optimization of Biological Control of Rhizoctonia Stem Rot of Poinsettia by *Paecilomyces lilacinus* and *Pseudomonas cepacia*

D. KELLY CARTWRIGHT, Former Graduate Research Assistant, and D. M. BENSON, Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27695

ABSTRACT

Cartwright, D. K., and Benson, D. M. 1995. Optimization of biological control of Rhizoctonia stem rot of poinsettia by *Paecilomyces lilacinus* and *Pseudomonas cepacia*. Plant Dis. 79:301-308.

Effects of antagonist concentration, nutrient status, and antagonist-free preparations (culture filtrate) of *Paecilomyces lilacinus* (isolate 6.2F) and *Pseudomonas cepacia* (strain 5.5B) on control of Rhizoctonia stem rot of poinsettia in polyfoam rooting cubes were investigated. Infection of cuttings ranged from 27 to 83% in cubes soaked in log 7.4 to log 3.4 conidia per milliliter of *P. lilacinus*. Percent infection of cuttings ranged from 0 to 56% in rooting cubes treated with *P. cepacia* applied in concentrations from log 9.4 to log 5.4 cfu per milliliter. Infection of cuttings in infested controls ranged from 87 to 93%. No significant differences in biocontrol occurred when *P. lilacinus* or *P. cepacia* were applied with or without a dilute potato-dextrose solution. There was no control (100% mortality) of stem rot with sterile culture filtrate from cultures of *P. cepacia*, but 70% control was achieved with filtrate from cultures of *P. lilacinus*. Both antagonists reduced the severity of Rhizoctonia stem rot on rooted cuttings in soilless medium. After 15–18 days, treatments with *P. lilacinus* had disease severity ratings (1 = healthy, 5 = dead) from 1.0 to 4.6 compared with infested or noninfested controls with ratings of 5.0 or 1.0, while ratings of poinsettia plants with all treatments of *P. cepacia* ranged from 2.3 to 3.8.

Continued problems associated with chemical pesticides, including fungicides, have stimulated interest in biocontrol strategies and the subsequent identification and characterization of antagonists useful for biocontrol purposes (1,3,11, 16,35,38,39). Some microorganisms, including a strain of *Streptomyces griseoviridis* (Mycostop, Kemira OY, Helsinki, Finland) for control of Fusarium diseases on carnation, *Gliocladium virens* (Glioguard, W. R. Grace and Co., Columbia, MD) for control of Pythium and Rhizoctonia diseases on greenhouse-grown crops, and a strain of *Pseudomonas cepacia* (Blue Circle, Stine Microbial Products, Madison, WI) for control of nematodes and Fusarium diseases on corn, are currently available or in the latter stages of commercial development (26,28,37). Many microorganisms with good potential, however, fail to be developed for practical use, primarily due to inconsistent performance associated with storing and using a living organism under changing and often adverse environmental conditions (2,17,33). In contrast, synthetic fungicides are usually reliable over a wider range of conditions. Several factors determine the success of an antagonist in biocontrol including the ability to compete with other microorganisms, unfavorable or fluctuating moisture levels, availability of nutrients, shelf-life, storage method, concentration of antagonist used, and timing and method of application. In addition, potential antagonists should be amenable for large-scale production and distribution, and not affect target or nontarget plants (2,7,29,33).

Stem rot of poinsettia (*Euphorbia*

Accepted for publication 19 December 1994.

© 1995 The American Phytopathological Society

pulcherrima Willd. ex Klotzsch), caused by *Rhizoctonia solani* Kühn (teleomorph = *Thanatephorus cucumeris* (A. B. Frank) Donk) is a serious disease of poinsettia during all stages of production (22,36). Many poinsettia cuttings are rooted in polyfoam, soilless, rooting cubes before transplanting to a potting medium. These cubes provide a conducive environment for infection of poinsettia and spread of *R. solani* and act as an inoculum source after cubes and cuttings are transplanted (5,14). Control of stem rot is dependent on cultural practices and chemical fungicides (32, 36), but the heightened constraints associated with fungicides, illustrated by the recent removal of benomyl from the ornamental market, have prompted searches for alternative methods of control.

Recently, biocontrol of stem rot in rooting cubes with *Paecilomyces lilacinus* (Thom) R. A. Samson (isolate 6.2F, NRRL 22772) and *Pseudomonas cepacia* (strain 5.5B, ATCC 55344) has been demonstrated (8). During propagation of poinsettias from cuttings, these antagonists provided excellent control of stem rot. However, mechanism(s) of action, method(s) of application, optimization of antagonist delivery system(s), and longevity of control after transplanting need investigation. The objectives of this research were to determine *1)* the optimum concentration of *P. lilacinus* and *P. cepacia* for Rhizoctonia stem rot control in rooting cubes, *2)* the effect of nutrient-rich or nutrient-poor antagonist preparations on efficacy of stem rot control in rooting cubes, *3)* the effect of cell-free filtrate of antagonists on stem rot control in rooting cubes, and *4)* the ability of *P. lilacinus* and *P. cepacia* to control stem rot after poinsettia cuttings are transplanted to a soilless medium.

MATERIALS AND METHODS

Greenhouse procedures. Poinsettia stock plants (cv. Gutbier V-14 Glory [red]) were maintained in 220 Metro mix (W. R. Grace Co., Cambridge, MA) contained in 6-L plastic pots on greenhouse benches. Plants were fertilized weekly with a commercial 20-20-20 (N-P-K) fertilizer (2.7 g/L). Magnesium sulfate (2.4 g/L) was applied as a drench each month. Every 3 mo, soil drenches of potassium nitrate (0.45 g/L), calcium nitrate (1.6 g/L), and a foliar spray of 1% molybdenum were applied. Plants were occasionally pruned to stimulate shoot growth.

Dry rooting cube strips (Oasis Rootcubes, Smithers-Oasis, Kent, Ohio) with individual cubes (25 × 51 mm long × 37 mm high [47 cm^3]) were soaked in plastic trays until saturation (40 ml per cube) with specific antagonist or control preparations. After soaking, cube strips (five individual cubes per strip = one replicate) were fitted with a styrofoam sleeve on the sides and bottom, secured with two rubber bands, and placed on greenhouse benches. After 18 to 24 h, a rice grain (twice-autoclaved rice; 25 g of rice per 18 ml of water) colonized by *R. solani* (isolate RS-3 from poinsettia, AG 4, NRRL 22805) for 4 to 9 days was placed on top of cube strips at the seam where individual cubes join about 2 cm from cuttings (six grains per strip). Poinsettia cuttings (5–8 cm in length) taken from stock plants were placed in the preformed cube holes on each side of a rice grain and immediately misted. A misting regime of 1 min of mist per hour, 14 times a day was used.

Effect of different concentrations of antagonists on Rhizoctonia stem rot control. Cultures of isolate 6.2F of *P. lilacinus* were obtained by streaking conidia/mycelium onto potato-dextrose agar (PDA) (Difco Laboratories, Detroit, MI). After 9–14 days at ambient temperature, the contents (fungal culture with agar) of one petri dish with mycelium/conidia only (mycelium removed from surface with minimal agar) from five to seven additional plates were blended in a Waring blender at high speed for 45–60 s in 600 ml of sterile, deionized water and strained through cheesecloth. This yielded a solution of approximately 0.1% PDA. This original preparation was serially diluted four times in 1:10 increments in sterile, 0.1% PDA solution. Concentration was determined with a hemacytometer and ranged from log 3.4 to log 7.4 conidia per milliliter.

Cultures of strain 5.5B of *P. cepacia* were obtained by streaking cells onto PDA and incubating plates at ambient temperature on a laboratory bench for 10–14 days. Preparations and concentrations were prepared as described above by blending contents (culture and agar) of a single petri dish with additional cultures (cells removed from surface with minimal agar) and serially diluting the original preparation in sterile, 0.1% PDA solution. Concentration ranged from log 5.4 to log 9.4 cfu per milliliter and was determined by plating 0.1 ml aliquots from dilutions onto King's medium B (KB) (12).

Effect of antagonists applied in a nutrient-rich or nutrient-poor suspension on Rhizoctonia stem rot control. Nutrient-rich preparations of *P. lilacinus* or *P. cepacia* were grown and prepared as stated in previous section. Nutrient-poor suspensions were obtained by removing cultures only (with minimal agar), suspending these in 600 ml of sterile, deionized water, and blending for 45 to 60 s. Cultures were removed by scraping colonies of *P. lilacinus* from agar surfaces with a clean, glass slide or rinsing *P. cepacia* with sterile, deionized water. Concentration averaged log 7.1 conidia per milliliter for *P. lilacinus* or log 9.4 cfu per milliliter for *P. cepacia*.

Effect of sterile, culture filtrate of antagonists on Rhizoctonia stem rot control. Both *P. lilacinus* and *P. cepacia* were grown, and nutrient-rich suspensions prepared as described in previous sections. Concentrations were log 7.2 for *P. lilacinus* and log 8.9 for *P. cepacia*. Propagule/cell-free preparations (filtrate) were prepared by centrifuging 600 ml of each antagonist suspension at 16,200 *g* for 10 min; the supernatant was then collected and vacuum filtered through a 0.45 μm filter followed by filtration through a 0.22 μm filter (Millipore, Bedford, MA) to ensure propagule/cell-free suspensions.

A randomized block design was used and experiments were repeated at least once. Three replicates with five cubes per replicate (15 total cuttings/treatment/experiment) were used for each treatment. Percent infection and mortality of plants were monitored daily by recording the number of diseased and dead cuttings. Infested and noninfested control cube strips were soaked in sterile, deionized water (water-based controls) or a 0.1% solution of PDA (nutrient-based controls). Rhizoctonia stem rot and cutting root development were assessed after 2 wk. Cuttings were rated for disease severity based on the following scale: 1 = no disease, 2 = lesions covering < 25% of stem, 3 = lesions covering 26 < 50% of stem, 4 = lesions covering 51 < 75% of stem, and 5 = stem completely girdled or collapsed. Root development was evaluated as follows: 0 = cutting collapsed, 1 = cutting with disease rating < 4, no root initials present, 1.5 = beginning of callus formation, 2 = distinct root initials, 2.5 = callus completely encircling the stem, 3 = callus tissue, beginning of lateral roots, 3.5 = callus plus distinct lateral roots. When possible, data were pooled based on homogeneity of variance. All data were analyzed with PC SAS (SAS Institute, Cary, NC) with PROC ANOVA or PROC GLM. Means were separated by the Waller-Duncan *k*-ratio test.

Application of antagonists to cubes for control of stem rot after transplanting. In some experiments, poinsettia cuttings rooted in the presence of *R. solani* and the antagonists were transplanted to a soilless medium to determine longevity of biocontrol. Nutrient-rich suspensions of *P. lilacinus* and *P. cepacia* were prepared as stated previously. Cube strips were soaked with suspensions and arranged in a randomized block design on greenhouse benches. Twelve replicates were used for all antagonist treatments and the noninfested, water-based control. Flutolanil (0.3 g a.i./L, NOR-AM, Wilmington, DE) was used as a fungicide control.

After 3 wk, cuttings were rated for disease severity based on the previously described scale. Root development was rated as follows: 0 = cutting collapsed,

1 = cutting with disease rating < 4, no root initials present, 2 = root initials (callus) present, plus beginning of lateral root formation, 3 = lateral roots extending through one outside cube surface, 4 = lateral roots extending through two outside cube surfaces, and 5 = roots extending through three outside cube surfaces. Experiments were repeated once or twice. Data were pooled based on homogeneity of variance, analyzed using PC SAS with PROC GLM, and means separated by the Waller-Duncan *k*-ratio test.

Biocontrol of Rhizoctonia stem rot after transplanting poinsettia cuttings to a soilless medium. Asymptomatic cuttings and the attached rooting cube from experiments described in the previous section were transplanted to a soilless medium (Metro mix 220) amended with either *P. lilacinus* or *P. cepacia*. To grow *P. lilacinus,* mycelial disks were taken from an actively growing colony and used to seed twice-autoclaved rice contained in 250-ml flasks. All subsequent cultures of *P. lilacinus* used for these tests were grown by seeding twice-autoclaved rice with *P. lilacinus*-colonized rice grains (≈50 colonized grains added to each flask of rice). Rice cultures of *P. lilacinus* were grown at ambient temperature for 12–14 days, pulverized at high speed in a Waring blender, and sifted through a 2-mm sieve. Sieved rice particles were suspended in sterile, deionized water and serially diluted (1:10) for quantification. Ten grams of rice particles colonized with *P. lilacinus* were used per 4 L of potting medium, and deionized water was added to bring soil moisture of the medium to ≈60% (w/w basis). *Pseudomonas cepacia* was grown, prepared, and quantified as stated in the previous sections. A liquid suspension containing cells of *P. cepacia* was added to the potting medium to yield ≈60% moisture. Both antagonist preparations were incorporated into potting medium by thorough mixing in plastic bags. After mixing, medium was triple bagged in clear, plastic bags (25 × 20 × 61 cm) and stored at room temperature. Bags were rotated every 2–3 days to ensure uniform growth and distribution of the antagonists. Concentration of antagonists was determined prior to their addition to the potting medium and was based on number of cfu per gram of bag-dry, soilless medium. After incubation periods of 7 or 14 days, populations of antagonists were assayed. For this, 1-g subsamples from 1-L samples of antagonist-infested potting medium were suspended in sterile, deionized water and serially diluted (0.1 ml aliquots plated). Acidified, one-half strength PDA (25 drops of 50% lactic acid solution per L of medium) was used for quantification of *P. lilacinus,* and KB medium or TB-T medium (15) for *P. cepacia.*

Inoculum of *R. solani* was grown on rice as described in previous sections. After 7–10 days, rice grains colonized with *R. solani* were pulverized at high speed in a Waring blender for 45–60 s followed by passage through a 2-mm sieve. Potting medium was placed in 10-cm-diameter clay pots (300 cm^3 of medium per pot). A 150 cm^3 portion of potting medium (antagonist-amended or unamended) was infested with 75 mg of sieved rice particles colonized by *R. solani.* This or a noninfested portion (150 cm^3) of medium was then added to the top of the pot (450 cm^3 of medium per pot total). Asymptomatic cuttings in individual polyfoam cubes were transplanted into the top 150 cm^3 of medium in each pot leaving about 5 mm of the cube top exposed. Cuttings were drenched with a commercial fertilizer (200 ppm N) and pots placed on a greenhouse bench. Pots were watered by drip irrigation (1 min irrigation, 5 times per day).

All pots (one pot = one replicate) were completely randomized on greenhouse benches. Treatments were based on a complete factorial design with four factors: *1)* antagonist present (*P. lilacinus* or *P. cepacia*); *2)* presence or absence of *R. solani* in the rooting cube; *3)* presence or absence of *R. solani* in potting medium; and *4)* incubation periods of 0, 7, or 14 days of antagonists in potting medium prior to transplanting, or unamended potting medium (control). There were three to five replicates per treatment. Poinsettia plants were monitored for 15–18 days. At the end of the test period, plants were rated for disease severity based on the previously stated scale. The experiment was repeated at least once. Analysis of the log of the variance (for treatment means) was performed with PROC GLM to test for similarity among experiments. Analysis of variance with PROC GLM was performed on treatment means of the 32 core treatments (involving antagonists) to test for interactions of the different factors. In addition, for clarity and presentation, antagonist treatments (*P. lilacinus* or *P. cepacia*) were analyzed separately and treatment means were compared with the infested control with the PDIFF option of the least-squares estimates with PROC GLM.

RESULTS

Effect of different concentrations of antagonists on Rhizoctonia stem rot control. After initial infection, Rhizoctonia stem rot progressed rapidly in all concentrations of isolate 6.2F of *P. lilacinus* and the infested controls with the exception of cuttings in cubes soaked in the original, undiluted preparation (highest concentration) of *P. lilacinus.* Both percent infection and mortality in the undiluted preparation averaged 27%, which was significantly less ($P = 0.05$) than infection of cuttings in all diluted concentrations in which infection ranged from 83 to 90% (Fig. 1). The average disease rating (2.1) of cuttings in the

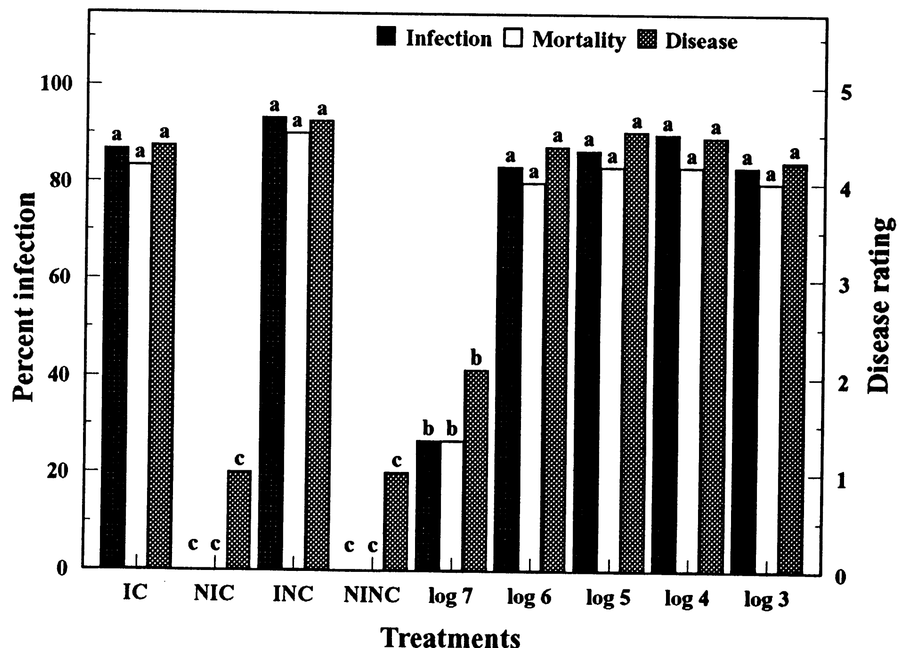

Fig. 1. Effect of different concentrations of *Paecilomyces lilacinus* (isolate 6.2F) on control of Rhizoctonia stem rot of poinsettia in polyfoam rooting cubes after 14 days. Values within each variable (infection, mortality, or disease rating) are significantly different ($P = 0.05$) if followed by a different letter according to the Waller-Duncan *k*-ratio test. Values are means from two representative experiments. Concentrations: log 7 = original, undiluted concentration (log 7.4 conidia per milliliter); log 6 = log 6.4 conidia per milliliter; log 5 = log 5.4 conidia per milliliter; log 4 = log 4.4 conidia per milliliter; log 3 = log 3.4 conidia per milliliter; IC = infested water-based control; NIC = noninfested water-based control; INC = infested nutrient-based control; NINC = noninfested nutrient-based control.

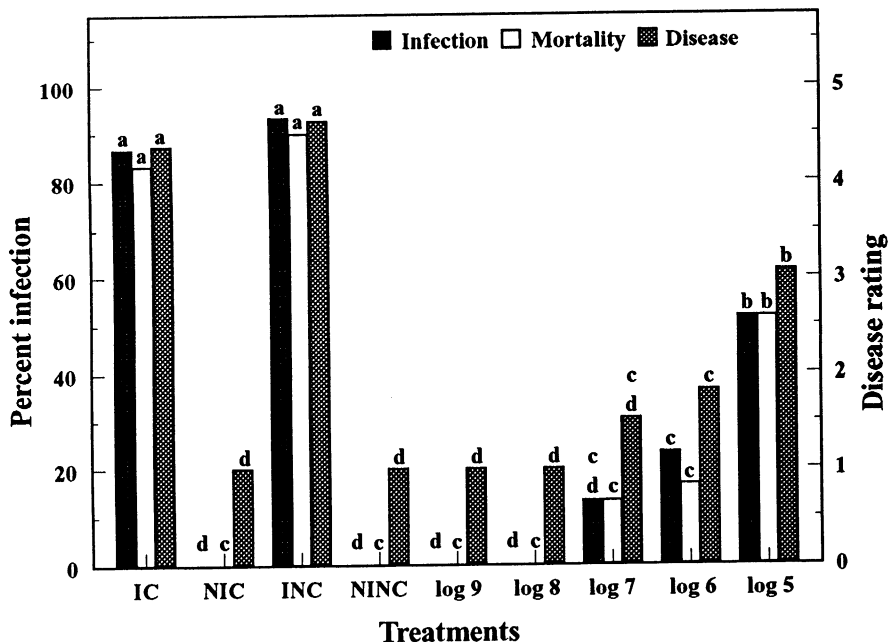

Fig. 2. Effect of different concentrations of *Pseudomonas cepacia* (strain 5.5B) on control of Rhizoctonia stem rot of poinsettia in polyfoam rooting cubes after 14 days. Values within each variable (infection, mortality, or disease rating) are significantly different ($P = 0.05$) if followed by a different letter according to the Waller-Duncan k-ratio test. Values are means from two representative experiments. Concentrations: log 9 = original, undiluted concentration (log 9.4 cfu per milliliter); log 8 = log 8.4 cfu per milliliter; log 7 = log 7.4 cfu per milliliter; log 6 = log 6.4 cfu per milliliter; log 5 = log 5.4 cfu per milliliter; IC = infested water-based control; NIC = noninfested water-based control; INC = infested nutrient-based control; NINC = noninfested nutrient-based control.

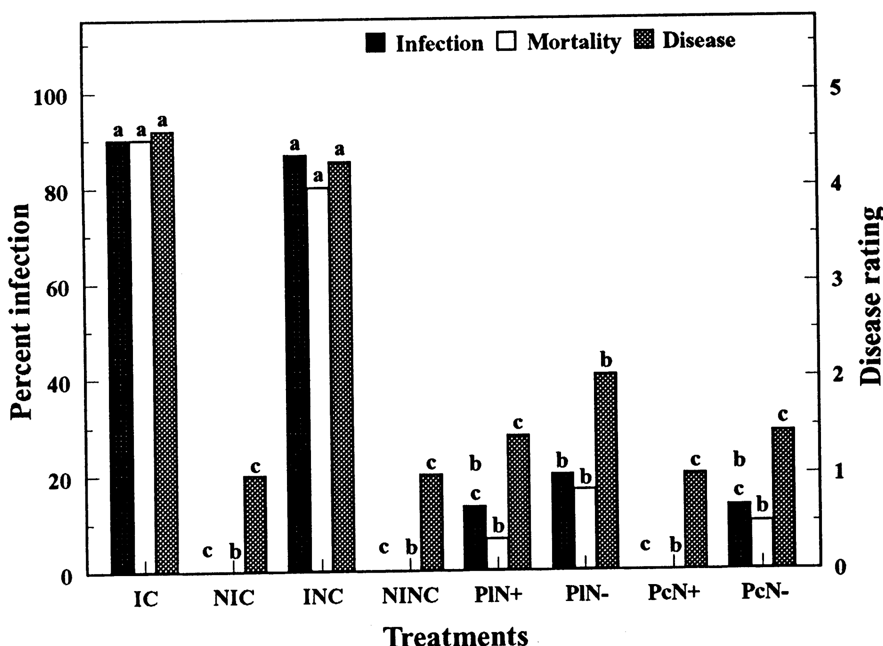

Fig. 3. Effect of *Paecilomyces lilacinus* (isolate 6.2F) or *Pseudomonas cepacia* (strain 5.5B) applied in a nutrient-rich or nutrient-poor suspension on control of Rhizoctonia stem rot of poinsettia in polyfoam rooting cubes after 14 days. Values within each variable (infection, mortality, or disease rating) are significantly different ($P = 0.05$) if followed by a different letter according to the Waller-Duncan k-ratio test. Values are means from two experiments. PIN+ = *P. lilacinus*, nutrient-rich; PIN− = *P. lilacinus*, nutrient-poor; PcN+ = *P. cepacia*, nutrient-rich; PcN− = *P. cepacia*, nutrient-poor; IC = infested water-based control; NIC = noninfested water-based control; INC = infested nutrient-based control; NINC = noninfested nutrient-based control.

highest concentration was significantly less than all other concentrations in which disease ratings ranged from 4.2 to 4.5, not different ($P = 0.05$) from infested controls (Fig. 1). Cuttings in the infested control (water-based) had the most developed roots (rating = 2.2), but were not significantly different from cuttings in the noninfested controls (1.9), infested, nutrient-based controls (1.5), or *P. lilacinus* at concentrations of log 5.4 or log 6.4 conidia per milliliter (*data not shown*). All other treatments had less root development.

No infection occurred with the two highest concentrations of *P. cepacia*. Compared with the infested controls, initial infection of cuttings in cubes treated with the log 7.4, log 6.4, or log 5.4 concentrations of *P. cepacia* was delayed after inoculation with *R. solani*. Infection of poinsettia cuttings ranged from 13 to 52% at the three lowest concentrations, respectively (Fig. 2). No infection occurred on cuttings in the noninfested controls. No significant differences in disease severity were found for cuttings in cubes treated with the three highest concentrations of *P. cepacia* and the noninfested controls. Cuttings in cubes soaked in the log 6.4 and log 5.4 cfu per milliliter preparations of *P. cepacia* had disease ratings of 1.8 and 3.1, which were significantly less than either infested control (Fig. 2). No significant difference in root development of poinsettia cuttings occurred among any concentration of *P. cepacia* (*data not shown*).

Effect of antagonists applied in a nutrient-rich or nutrient-poor suspension on Rhizoctonia stem rot control. Some infection of cuttings occurred with all nutrient-rich and nutrient-poor treatments except with *P. cepacia* applied in a nutrient-rich preparation (Fig. 3). Initial infection and progress with all antagonist treatments prepared in nutrient-rich or nutrient-poor solutions, however, was delayed compared with infested controls. Percent infection (13–20%) and mortality (7–17%) of cuttings in cubes treated with *P. lilacinus* were not significantly different regardless of nutrient status (Fig. 3). Percent infection and mortality of cuttings in cubes treated with *P. cepacia* under nutrient-poor conditions was 13 and 10%, respectively. Infection and mortality of cuttings in the infested controls ranged from 80 to 90% (Fig. 3). No infection or mortality occurred on cuttings in noninfested controls. There was a significant difference in disease severity with nutrient-rich preparations of *P. lilacinus* compared with nutrient-poor preparations. No difference ($P = 0.05$) in disease severity occurred between treatments with *P. cepacia* in nutrient-rich or nutrient-poor preparations (Fig. 3). Cuttings in all treatments with antagonists had significantly less disease than cuttings in

infested controls. No significant differences in root development occurred between treatments (*data not shown*).

Effect of sterile, culture filtrate of antagonists on Rhizoctonia stem rot control. Over a 2-wk period, Rhizoctonia stem rot progress on cuttings was limited with both propagule-free and propagule-containing preparations of *P. lilacinus* and cell-containing preparations of *P. cepacia*. Infection and mortality of cuttings in cubes treated with preparations of *P. lilacinus* were not significantly different from noninfested controls in which no infection occurred (Fig. 4). Propagule-free preparations of *P. lilacinus* limited infection and mortality to 30%, significantly more than propagule-containing preparations but less ($P = 0.05$) than infested controls in which infection and mortality ranged from 97 to 100% (Fig. 4). No differences in root development occurred with any treatment (*data not shown*).

Percent infection and mortality in preparations containing cells of *P. cepacia* were 17 and 13%, respectively (Fig. 4). No control (100% infection) was obtained using cell-free preparations of *P. cepacia* (Fig. 4). Disease severity was also less ($P = 0.05$) on cuttings in cubes treated with cell-containing preparations of *P. cepacia* compared with cell-free filtrate or infested control treatments (Fig. 4). Disease severity ratings ranged from 4.9 to 5.0 for the infested controls and the cell-free preparations of *P. cepacia* (Fig. 4). Root development was not significantly different among any treatments (*data not shown*).

Biocontrol of Rhizoctonia stem rot after transplanting poinsettia cuttings to a soilless medium. Transplanted to a soilless medium, poinsettia plants in most antagonist treatments, regardless of factor or combination, had less disease ($P = 0.05$) compared with the infested control (noninfested, untreated cubes in infested, unamended soilless medium) (disease rating = 5.0) (IC) (Figs. 5,6). Disease control was variable on plants in treatments with isolate 6.2F of *P. lilacinus* (Fig. 5). However, all plants in treatments utilizing *P. lilacinus* had less ($P = 0.05$) disease than the infested control (rating = 5.0) except those in which the cubes were infested, treated with *P. lilacinus*, and transplanted to infested, soilless medium unamended or incubated with *P. lilacinus* for 14 days (ratings = 3.8,4.6) (Fig. 5). The best treatments with *P. lilacinus* had plants with average disease ratings of 1.0, 1.3, 1.4, or 1.8 (Fig. 5). No disease occurred on plants in the noninfested, *P. lilacinus*-treated cubes that were transplanted to noninfested medium with or without *P. lilacinus* (Fig. 5). After transplanting, disease ratings of poinsettia plants for all combinations of *P. cepacia* and *R. solani* in cubes or soilless medium ranged from 2.3 to 3.8 (Fig. 6). No disease

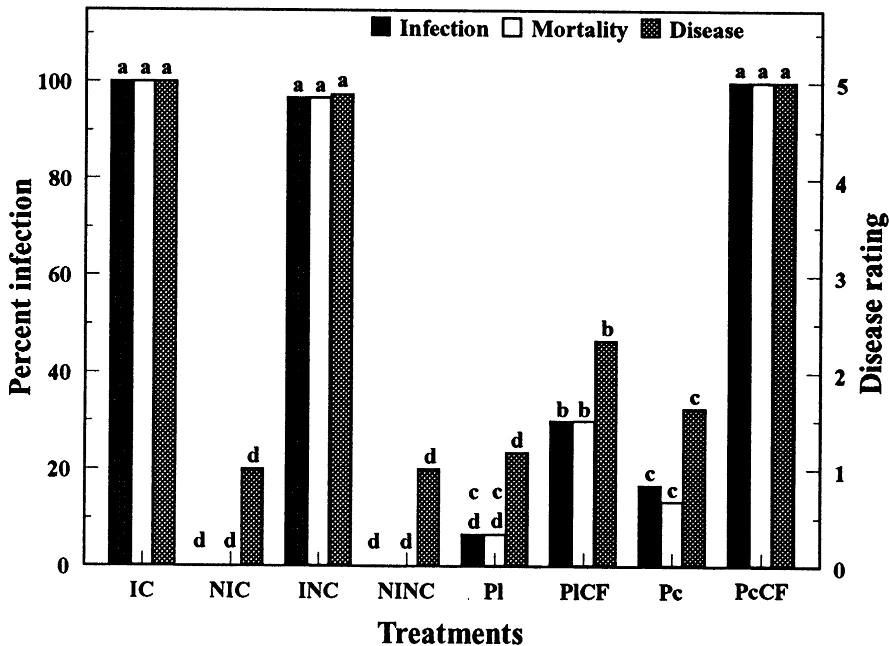

Fig. 4. Effect of culture filtrate of *Paecilomyces lilacinus* (isolate 6.2F) or *Pseudomonas cepacia* (strain 5.5B) on control of Rhizoctonia stem rot of poinsettia in polyfoam rooting cubes after 14 days. Values within each variable (infection, mortality, or disease rating) are significantly different ($P = 0.05$) if followed by a different letter according to the Waller-Duncan k-ratio test. Values are means from two experiments. Pl = *P. lilacinus*; PlCF = propagule-free filtrate of *P. lilacinus*; Pc = *P. cepacia*; PcCF = cell-free filtrate of *P. cepacia*; IC = infested water-based control; NIC = noninfested water-based control; INC = infested nutrient-based control; NINC = noninfested nutrient-based control.

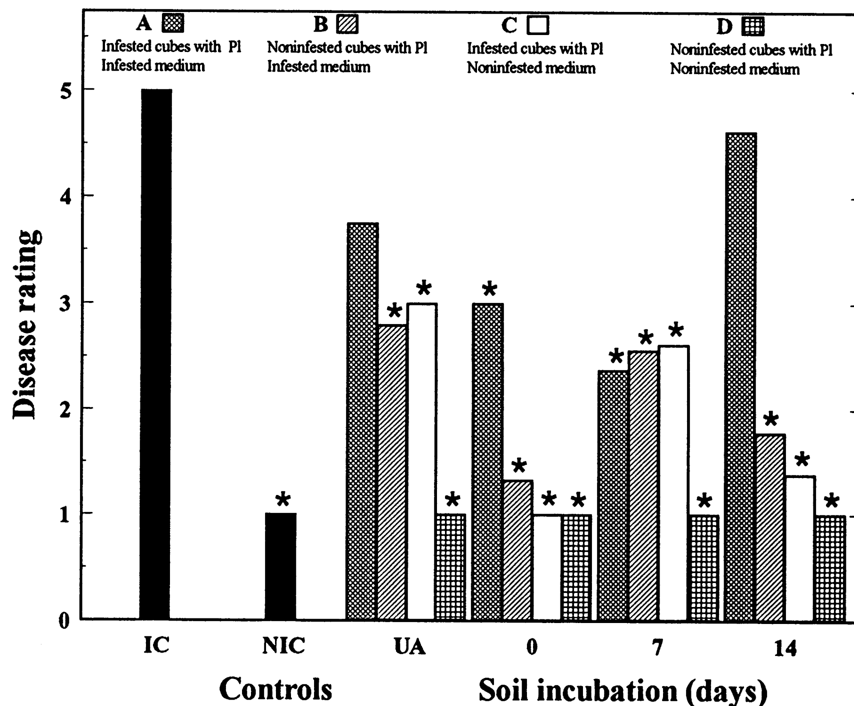

Fig. 5. Disease development on poinsettia cuttings rooted in polyfoam rooting cubes after transplanting to a soilless potting medium incubated with *Paecilomyces lilacinus* (isolate 6.2F) for 0, 7, or 14 days. A = cuttings in cubes treated with *P. lilacinus*, infested with *R. solani*, and transplanted to soilless medium infested with *R. solani*; B = cuttings in noninfested cubes treated with *P. lilacinus* and transplanted to soilless medium infested with *R. solani*; C = cuttings in cubes treated with *P. lilacinus*, infested with *R. solani*, and transplanted to noninfested, soilless medium; D = cuttings in noninfested cubes treated with *P. lilacinus* and transplanted to noninfested, soilless medium. Means from each treatment are significantly different ($P = 0.05$) from the mean of the infested control (IC) if marked by an asterisk, based on the least-squares estimate of the means. Values are means from two experiments. IC = cuttings in noninfested, untreated cubes transplanted to unamended soilless medium infested with *R. solani*; NIC = cuttings in noninfested, untreated cubes transplanted to unamended, noninfested soilless medium; UA = unamended (no *P. lilacinus* in soilless potting medium).

occurred on plants rooted in noninfested, *P. cepacia*-treated cubes and transplanted to noninfested medium with or without *P. cepacia* (Fig. 6). No disease occurred in the noninfested, untreated control and only limited disease occurred in the fungicide treatment (*data not shown*).

The only significant effects involved the presence or absence of *R. solani* in the cubes or potting medium (Table 1). The level of disease due to the presence or absence of the pathogen in the cube or soil was not dependent on which antagonist was used. In addition, there was no significant interaction between the incubation periods or the unamended soilless medium (by antagonist).

Both antagonists survived well when incubated in soilless potting medium at room temperature. The population of *P. lilacinus* was generally higher after incubation while the population of *P. cepacia* increased slightly after 7 days and then decreased slightly after 14 days incubation (Fig. 7A,B).

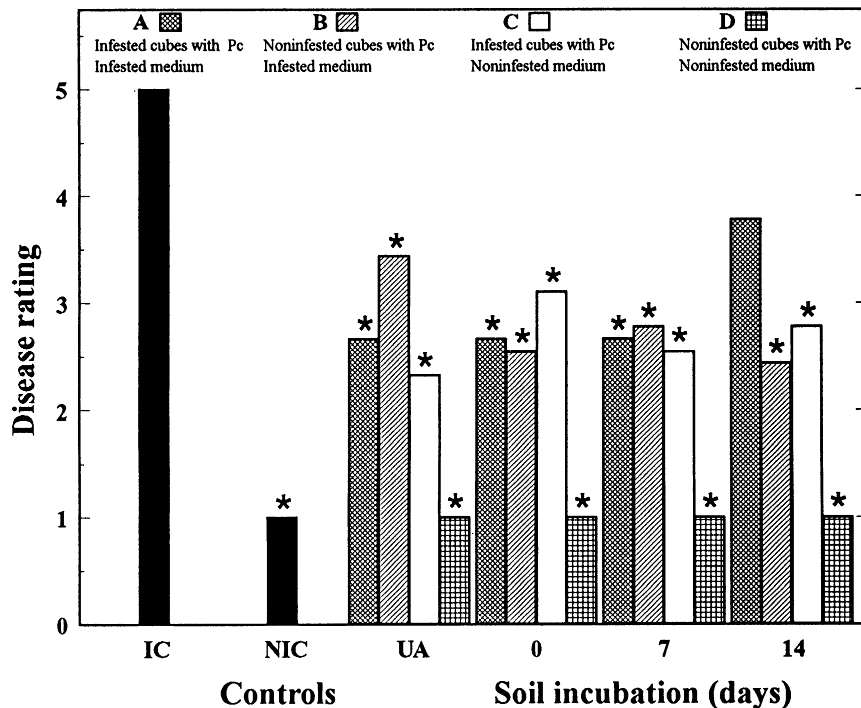

Fig. 6. Disease development on poinsettia cuttings rooted in polyfoam rooting cubes after transplanting to a soilless potting medium incubated with *Pseudomonas cepacia* (strain 5.5B) for 0, 7, or 14 days. A = cuttings in cubes treated with *P. cepacia*, infested with *R. solani*, and transplanted to soilless medium infested with *R. solani*; B = cuttings in noninfested cubes treated with *P. cepacia* and transplanted to soilless medium infested with *R. solani*; C = cuttings in cubes treated with *P. cepacia*, infested with *R. solani*, and transplanted to noninfested, soilless medium; D = cuttings in noninfested cubes treated with *P. cepacia* and transplanted to noninfested, soilless medium. Means from each treatment are significantly different ($P = 0.05$) from the mean of the infested control (IC) if marked by an asterisk, based on the least-squares estimate of the means. Values are means from two experiments. IC = cuttings in noninfested, untreated cubes transplanted to unamended soilless medium infested with *R. solani*; NIC = cuttings in noninfested, untreated cubes transplanted to unamended, noninfested soilless medium; UA = unamended (no *P. cepacia* in soilless potting medium).

Table 1. Effects of using *Paecilomyces lilacinus* (isolate 6.2F) or *Pseudomonas cepacia* (strain 5.5B) incubated for different periods in soilless potting medium to control Rhizoctonia stem rot of poinsettia in potting medium[a]

Source[b]	Degrees of freedom	Mean square	*F*	*Pr* > *F*
Experiment	1	0.02990017	0.03	0.8745
Antagonist	1	0.69792101	0.59	0.4475
RS-3 in cube	1	18.61562934	15.17	0.0004
Antagonist × RS-3 in cube	1	0.39323351	0.33	0.5678
RS-3 in potting medium	1	19.04958767	16.15	0.0003
Antagonist × RS-3 in potting medium	1	0.00073351	0.00	0.9803
RS-3 in cube × RS-3 in potting medium	1	1.81687934	1.54	0.2238
Antagonist × RS-3 in cube × RS-3 in potting medium	1	2.21885851	1.88	0.1800
Incubation (antagonist)	6	0.70649161	0.60	0.7288
RS-3 in cube × incubation (antagonist)	6	0.45506800	0.39	0.8824
RS-3 in potting medium × incubation (antagonist)	6	0.56572772	0.48	0.8182
RS-3 in cube × RS-3 in potting medium × incubation (antagonist)	6	1.38149161	1.17	0.3467

[a] Data pooled from two experiments.
[b] Antagonist = *P. lilacinus* or *P. cepacia*; RS-3 = *Rhizoctonia solani*; incubation = antagonists incubated in potting medium for 0, 7, or 14 days or unamended (antagonist-free).

DISCUSSION

Development of biocontrol agents requires the elucidation of characteristics such as mechanism(s) of action, optimum rate(s) and concentration(s) of antagonist applied to target area(s), carrier or preparation substrate, and method(s) of application (7,11,18,24,30).

Our results demonstrate that concentration of isolate 6.2F of *P. lilacinus* has a significant effect on control of Rhizoctonia stem rot of poinsettia in rooting cubes. Only the highest concentration (log 7 cfu per milliliter) gave acceptable control. The lack of control with lower concentrations could pose limitations, especially if isolate 6.2F is difficult to grow under large-scale production (33). The reason for the lack of control at lower concentrations is unknown but may be due to dilution of compound(s) inhibitory to *R. solani* during suspension preparation. This isolate does produce inhibitory compounds to *R. solani* in vitro (D. K. Cartwright and D. M. Benson, *unpublished data*). Other isolates of *P. lilacinus* have been shown to produce antifungal compounds (13). The effectiveness of propagule-free culture filtrate of isolate 6.2F in controlling stem rot suggests that inhibitory compound(s) were present in the filtrate suspension.

The addition of nutrients is important when applying some fungi for biocontrol, including *P. lilacinus* (6,23,27). Although nutrient status of preparations of isolate 6.2F had no significant effect on control, infection, mortality, and disease severity were typically higher over a 2-wk period when nutrient-poor preparations were used than when nutrient-rich preparations were used. The effect of adding specific nutrients to enhance antibiotic production needs evaluation.

Antagonist concentration can be an important factor in efficacy of disease control with bacterial antagonists (18). Significant control of stem rot was achieved with all concentrations of strain 5.5B of *P. cepacia* even though effectiveness decreased as concentration decreased. The efficacy of a wider range of concentrations is important from an application, economic, and ecological perspective. Application methods are more adaptable, time required for the bacterium to reach necessary levels of concentration is reduced during fermentation, and timing and conditions may not be as critical when applying the bacterium to the target area(s).

Nutrient status did not have a marked effect on performance of strain 5.5B in these experiments. However, disease control was slightly less over a 2-wk period when nutrient-poor preparations of strain 5.5B were used. This may be due to the effect of nutrients on production of antibiotic(s) or on survivability of the bacterium in the cubes. As in the case of fungal antagonists, however, the role of specific nutrients in enhancing production of antibiotic compounds and possibly increasing efficacy of control needs investigation.

Cell-free filtrate of *P. cepacia* gave no control (100% mortality) of stem rot. Compounds inhibitory to *R. solani*, including pyrrolnitrin, have been isolated and characterized from cultures of strain 5.5B (10). However, inhibitory compounds were not present in the filtrate or were ineffective in the rooting cubes. Antibiotic compound(s) could remain within cells of *P. cepacia*, not being released until proper conditions prevail, the bacterial cell lyses, or both. Howell and Stipanovich (21) demonstrated that cells of *Pseudomonas fluorescens* released pyrrolnitrin as cells lysed, resulting in protection of cotton seedlings from *R. solani*. The population of strain 5.5B does decline rapidly in the rooting cubes (9). More experiments are necessary to determine the exact role of antibiotics in stem rot control, an important mechanism with some bacterial antagonists (20,34), although other research has shown that antibiotics play a minimal role in biocontrol, with mechanisms such as competition for nutrients or space being more critical (25,28,31).

If antibiosis is the mode of action for these antagonists, it could be a desirable mechanism of action for use in this particular system. The dry, polyfoam cubes are very liquid absorbent, allowing for complete and uniform distribution of a liquid suspension, eliminating the need for cube colonization by antagonists. In addition, the nature of the cube matrix makes it conducive to the addition of selective amendments to augment or enhance antibiotic production.

Both antagonists have potential to control Rhizoctonia stem rot after poinsettia cuttings are transplanted. Most treatments with *P. lilacinus* or *P. cepacia* limited stem rot severity on poinsettia transplants although the level of control with most treatments was not commercially acceptable. However, complete control of stem rot was achieved with some treatments using isolate 6.2F of *P. lilacinus*. Because of this, further investigation using isolate 6.2F to control stem rot in soilless medium is warranted.

Control of stem rot with strain 5.5B of *P. cepacia* after transplanting was considerably less than the high level of control achieved in rooting cubes (8). The most effective control was achieved in treatments in which strain 5.5B and *R. solani* were added to rooting cubes, and then the plants were transplanted to noninfested, unamended soilless medium. This control, however, was similar to other treatments in which combinations of the antagonist/pathogen in the cube and antagonist/pathogen in the soil were used. No marked improvement was attained and no trend in control was evident regardless of incubation period of the bacterium in the soilless medium. Reapplication of strain 5.5B to rooting cubes during the rooting period might improve control after transplanting.

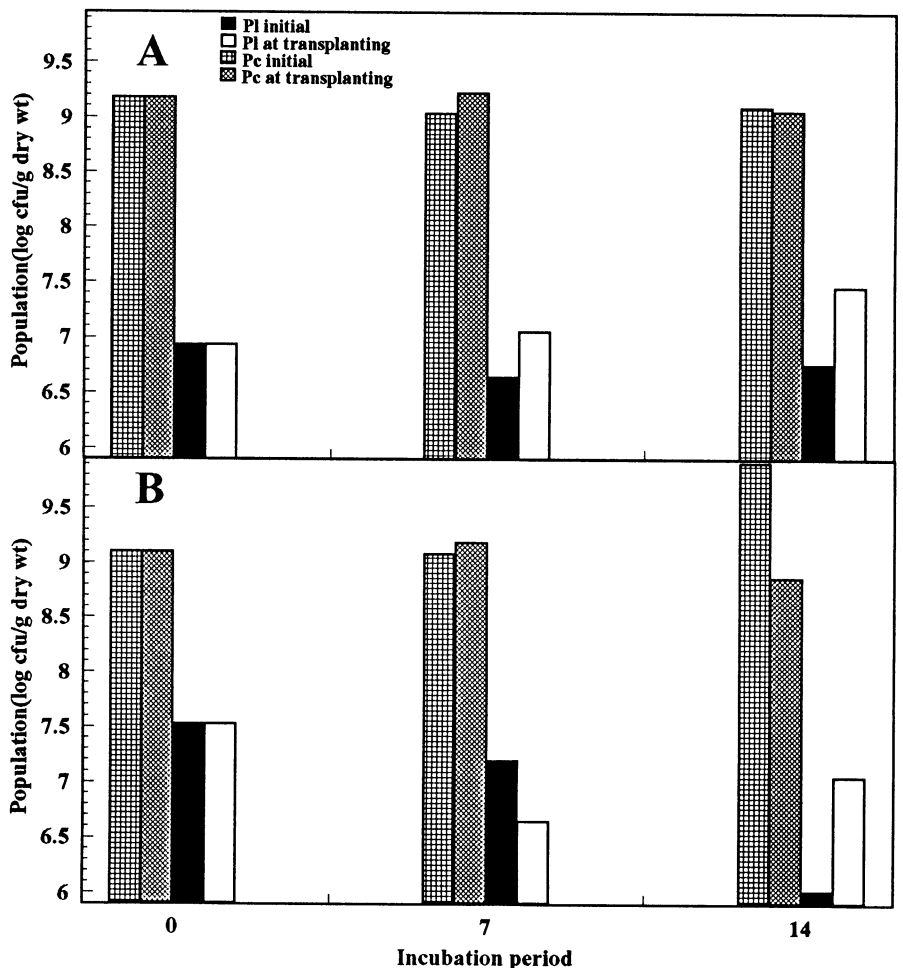

Fig. 7. Population of isolate 6.2F of *Paecilomyces lilacinus* or strain 5.5B of *Pseudomonas cepacia* initially applied to soilless potting medium and at transplanting (incubated for 0, 7, or 14 days). **(A)** population counts for experiment one; **(B)** population counts for experiment two.

Our data show that the population of *P. lilacinus* typically increased after incubation for 7 or 14 days. In rooting cubes, *P. lilacinus* was stable, although the population did decrease slightly after 3 wk (9). In other studies, populations of *P. lilacinus* used to control *Meloidogyne incognita* on tomato increased as much as 11-fold (6). Other antagonistic fungi, including *Trichoderma* spp. and *G. virens* increased by 100-fold in soil planted with cotton or left unplanted (4). Survival of strain 5.5B of *P. cepacia* in rooting cubes was limited and appears subject to a carrying capacity dictated by the cube environment (9). When incubated in a soilless medium, however, survival of strain 5.5B was greater. In these tests, the population increased or decreased only slightly from the initial population over a 7- or 14-day period. Some strains of *P. cepacia* survive well after application to target areas. Hebbar et al (19) demonstrated that populations of *P. cepacia* applied to corn roots increased on both the roots and in the rhizosphere. Parke (31) showed that populations of *P. cepacia* used to control damping-off of peas increased, particularly if low initial densities of the bacterium were applied.

ACKNOWLEDGMENTS

The authors wish to thank Billy I. Daughtry for technical assistance and Marcia Gumpertz, Dept. of Statistics, for assistance with statistical analysis. The authors also thank Smithers-Oasis U.S.A., Kent, OH, for providing Rootcubes, and Fairview Greenhouses and Garden Center, Raleigh, NC, for providing poinsettia stock plants. This research was supported, in part, by the North Carolina Agricultural Research Service, North Carolina State University, Raleigh, and by a grant from Ciba Geigy Agricultural Division, Greensboro, NC.

LITERATURE CITED

1. Baker, K. F. 1983. The future of biological and cultural control of plant disease. Pages 422-430 in: Challenging Problems in Plant Health. T. Kommedahl and P. H. Williams, eds. American Phytopathological Society, St. Paul, MN.
2. Baker, K. F. and Cook, R. J. 1974. Biological

control in plant pathology. Pages 50-55 in: Biological Control of Plant Pathogens. K. F. Baker and R. J. Cook, eds. W. H. Freeman and Company, San Francisco.

3. Baker, K. F. and Cook, R. J. 1974. Why biological control? Pages 343-348 in: Biological Control of Plant Pathogens. K. F. Baker and R. J. Cook, eds. W. H. Freeman and Company, San Francisco.
4. Beagle-Ristaino, J. E., and Papavizas, G. C. 1985. Survival and proliferation of propagules of *Trichoderma* spp. and *Gliocladium virens* in soil and in plant rhizospheres. Phytopathology 75:729-732.
5. Benson, D. M. 1991. Control of Rhizoctonia stem rot of poinsettia during propagation with fungicides that prevent colonization of rooting cubes by *Rhizoctonia solani*. Plant Dis. 75:394-398.
6. Cabanillas, E., Barker, K. R., and Nelson, L. A. 1989. Survival of *Paecilomyces lilacinus* in selected carriers and related effects on *Meloidogyne incognita* on tomato. J. Nematol. 21:121-130.
7. Campbell, R. 1989. Biological Control of Microbial Plant Pathogens. Cambridge University Press, Cambridge.
8. Cartwright, D. K., and Benson, D. M. 1995. Biological control of Rhizoctonia stem rot of poinsettia in polyfoam rooting cubes with *Pseudomonas cepacia* and *Paecilomyces lilacinus*. Biol. Control. (In press.)
9. Cartwright, D. K., and Benson, D. M. 1994. Effect of population dynamics of *Pseudomonas cepacia* and *Paecilomyces lilacinus* in polyfoam rooting cubes in relation to colonization by *Rhizoctonia solani*. Appl. Environ. Microbiol. 60:2852-2857.
10. Cartwright, D. K., Chilton, W. S., and Benson, D. M. 1995. Pyrrolnitrin and phenazine production by *Pseudomonas cepacia*, strain 5.5B, a biocontrol agent of *Rhizoctonia solani*. Appl. Microbiol. Biotechnol. (In press.)
11. Cook, R. J., and Baker, K. F. 1983. Perspectives. Pages 426-444 in: The Nature and Practice of Biological Control of Plant Pathogens. R. J. Cook and K. F. Baker, eds. American Phytopathological Society, St. Paul, MN.
12. Dhingra, O. D., and Sinclair, J. B. 1985. Basic Plant Pathology Methods. CRC Press, Inc. Boca Raton, FL.
13. Domsch, K. H., Gams, W., and Anderson, T. H. 1980. *Paecilomyces lilacinus*. Pages 530-532 in: A Compendium of Selected Soil Fungi, vol. 1. Academic Press, NY.
14. Ecke, P., Jr., Matkin, O. A., and Hartley, D. E., eds. 1990. The Poinsettia Manual. Paul Ecke Poinsettias, Encinitas, CA.
15. Hagedorn, C., Gould, W. D., Bardinelli, T. R., and Gustavson, D. R. 1987. A selective medium for enumeration and recovery of *Pseudomonas cepacia* biotypes from soil. Appl. Environ. Microbiol. 53:2265-2268.
16. Hallberg, G. R. 1987. Agricultural chemicals in groundwater: Extent and implication. Am. J. Alt. Agri. 2:3-15.
17. Handelsman, J., and Parke, J. L. 1989. Mechanisms in biocontrol of soilborne plant pathogens. Pages 27-61 in: Plant-Microbe Interactions: Molecular and Genetic Perspectives, vol. 3. T. Kosuge and E. W. Nester, eds. McGraw-Hill, New York.
18. Hebbar, K. P., Atkinson, D., Tucker, W., and Dart, P. J. 1992. *Pseudomonas cepacia*, a potential suppressor of maize soil-borne diseases - seed inoculation and maize root colonization. Soil Biol. Biochem. 24:999-1007.
19. Hebbar, K. P., Atkinson, D., Tucker, W., and Dart, P. J. 1992. Suppression of *Fusarium moniliforme* by maize root-associated *Pseudomonas cepacia*. Soil Biol. Biochem. 24:1009-1020.
20. Homma, Y., and Suzui, T. 1989. Role of antibiotic production in suppression of radish damping-off by seed bacterization with *Pseudomonas cepacia*. Ann. Phytopath. Soc. Jpn. 55:643-652.
21. Howell, C. R., and Stipanovic, R. D. 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology 69:480-482.
22. Jones, R. K. 1990. Poinsettia diseases and their management. N.C. Flow. Grow. Bull. 35:6-11.
23. Jones, R. W., Pettit, R. E., and Taber, R. A. 1984. Lignite and stillage: Carrier and substrate for application of fungal biocontrol agents to the soil. Phytopathology 74:1167-1170.
24. Knudsen, G. R., and Spurr, H. W., Jr. 1988. Management of bacterial populations for foliar disease biocontrol. Pages 83-92 in: Biocontrol of Plant Diseases, vol. 1. K. G. Mukerji and K. L. Garg, eds. CRC Press, Inc., Boca Raton, FL.
25. Kraus, J., and Loper, J. E. 1992. Lack of evidence for a role of antifungal metabolite production by *Pseudomonas fluorescens* Pf-5 in biological control of Pythium damping-off of cucumber. Phytopathology 82:264-271.
26. Lumsden, R. D., and Locke, J. C. 1989. Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* with *Gliocladium virens* in soilless mix. Phytopathology 79:361-366.
27. Lumsden, R. D., Locke, J. C., Adkins, S. T., Walter, J. F., and Ridout, C. J. 1992. Isolation and localization of the antibiotic gliotoxin produced by *Gliocladium virens* from alginate prill in soil and soilless media. Phytopathology 82:230-235.
28. McLoughlin, T. J., Quinn, J. P., Bettermann, A., and Bookland, R. 1992. *Pseudomonas cepacia* suppression of sunflower wilt fungus and role of antifungal compounds in controlling the disease. Appl. Environ. Microbiol. 58:1760-1763.
29. Merriman, P., and Russell, K. 1990. Screening strategies for biological control. Pages 427-435 in: Biological Control of Soil-borne Plant Pathogens. D. Hornby, ed. CAB International, Wallingford, Oxon, UK.
30. Morris, C. E., and Rouse, D. I. 1985. Role of nutrients in regulating epiphytic bacterial populations. Pages 63-82 in: Biological Control on the Phylloplane. C. E. Windels and S. E. Lindow, eds. American Phytopathological Society, St. Paul, MN.
31. Parke, J. L. 1990. Population dynamics of *Pseudomonas cepacia* in the pea spermosphere in relation to biocontrol of *Pythium*. Phytopathology 80:1307-1311.
32. Powell, C. C., Jr. 1988. The safety and efficacy of fungicides for use in Rhizoctonia crown rot control of directly potted unrooted poinsettia cuttings. Plant Dis. 72:693-695.
33. Powell, K. A., Faull, J. L., and Renwick, A. 1990. The commercial and regulatory challenge. Pages 445-464 in: Biological Control of Soil-borne Plant Pathogens. D. Hornby, ed. CAB International, Wallingford, Oxon, UK.
34. Smilanick, J. L., and Denis-Arrue, R. 1992. Control of green mold of lemons with *Pseudomonas* species. Plant Dis. 76:481-485.
35. Staub, T., and Sozzi, D. 1984. Fungicide resistance: A continuing challenge. Plant Dis. 68:1026-1031.
36. Strider, D. L., and Jones, R. K. 1985. Poinsettias. Pages 351-403 in: Diseases of Floral Crops, vol. 2. D. L. Strider, ed. Praeger Publishers, New York.
37. Tahvonen, R. and Avikaninen, H. 1987. The biological control of seed-borne *Alternaria brassicicola* of cruciferous plants with a powdery preparation of *Streptomyces* sp. J. Agri. Sci. Finl. 59:199-208.
38. Tweedy, B. G. 1983. The future of chemicals for controlling plant diseases. Pages 405-415 in: Challenging Problems in Plant Health. T. Kommedahl and P. H. Williams, eds. American Phytopathological Society, St. Paul, MN.
39. Urbain, C. D. 1986. The chemical war heats up. Farm J. 110:15-16.