PROOF Biological Control of Pythium Root Rot of Chrysanthemum in Small-scale Hydroponic Units

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The capacity of several strains of root-colonizing bacteria to suppress *Pythium aphanidermatum*, *Pythium dissotocum* and root rot was investigated in chrysanthemums grown in single-plant hydroponic units containing an aerated nutrient solution. The strains were applied in the nutrient solution at a final density of 10^4 CFU ml^{-1} and 14 days later the root systems were inoculated with *Pythium* by immersion in suspensions of 10^4 zoospores ml^{-1} solution. Controls received no bacteria, no *Pythium*, or one of the *Pythium* spp. but no bacteria. Strain effectiveness was estimated based on percent roots colonized by *Pythium* and area under disease progress curves (AUDPC). In plants treated respectively with *Pseudomonas* (*Ps.*) *chlororaphis* 63-28 and *Bacillus cereus* HY06 and inoculated with *P. aphanidermatum*, root colonization by the pathogen was 83% and 72% lower than in the pathogen control, and AUDPC value was reduced by 61% and 65%. For *P. dissotocum*, the respective strains reduced root colonization by 87% and 91%, and AUDPC values by 70% and 90%. In plants treated respectively with *Pseudomonas chlororaphis* Tx-1 and *Comamonas acidovorans* c-4-7-28, root colonization by *P. aphanidermatum* was 84% and 80% lower than in the controls and AUDPC values were reduced by 66% and 57%; these strains did not suppress *P. dissotocum*. *Burkholderia gladioli* C-2-74 and *C. acidovorans* OCR-7-8-38, respectively, suppressed colonization of roots by *P. dissotocum* by 74% and 86%, and reduced AUDPC values by 60% and 70%, but were ineffective against *P. aphanidermatum*. *C. acidovorans* OCR-7-8-39 reduced colonization and AUDPC values of *P. aphanidermatum* by 57% and 42%, respectively. *Pseudomonas corrugata* 13, *Ps. fluorescens* 15 and JZ12, and three additional strains of *C. acidovorans* were weakly or nonsuppressive against *P. aphanidermatum.* Strains that reduced AUDPC values for *P. aphanidermatum* or *P. dissotocum* when applied at 10^4 CFU ml^{-1} were 11%–39% less effective at 10^3 CFU ml^{-1}. Four tested strains (*Ps. chlororaphis* 63-28, *Ps. chlororaphis* Tx-1, *B. cereus* HY06, and *B. gladioli* C-7-24) in most instances suppressed root colonization and lowered AUDPC values of *P. aphanidermatum* when applied at 14, 7 or 0 days before inoculation, but reduction of the respective variables was generally greater when the strains were applied at 14 days (63%–87% and 75%–78%) or 7 days (44%–47% and 31%–88%) than at 0 days (14%–31% and 23%–62%) before inoculation. *Ps. chlororaphis* Tx-1, *Ps. chlororaphis* 63-28 and *B. cereus* HY06 significantly suppressed *P. aphanidermatum* whether the temperature of the nutrient solution was high (32°C) or moderate (24°C). Taken together, the observations suggest that *Ps. chlororaphis* 63-28, *B. cereus* HY06, *Ps. chlororaphis* Tx-1, *B. gladioli* C-2-74 and *C. acidovorans* OCR-7-8-38 have the potential for controlling Pythium root rot in hydroponic chrysanthemums.

KEY WORDS: Biological control; *Chrysanthemum morifolium*; hydroponic; Pythium root rot.

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INTRODUCTION

Hydroponic systems have considerable potential for increasing production efficiency of cut chrysanthemums (*Chrysanthemum morifolium* L.) in greenhouses, compared with conventional production methods in ground beds (I. Smith, Flowers Canada [Ontario] Inc.). However, in feasibility tests of hydroponic production conducted by a commercial grower in Ontario, root rot seriously constrained production of marketable chrysanthemums. The tests employed plastic troughs with coconut fiber or no rooting medium, through which nutrient solution was recirculated from reservoirs. *Pythium aphanidermatum* (Edson) Fitzp. and *Pythium dissotocum* Drechsler were consistently recovered from the roots of affected plants, which exhibited tip necrosis, discoloration (browning or graying), and progressive root rot (H. Yu, W. Liu and J.C. Sutton, 2001, unpublished observations). It was concluded that adoption of the hydroponic trough systems for commercial production of chrysanthemums would not be feasible unless Pythium root rot could be effectively controlled.

Management practices against Pythium root rot in hydroponic crops include measures to reduce or prevent introduction of *Pythium* inoculum into the greenhouse, sanitation to destroy or remove inoculum in sources within the greenhouse, and methods to suppress inoculum production and dispersal and disease increase in the crop (26). Practices to exclude or destroy *Pythium* inoculum in greenhouses normally are not sufficiently effective, in part because *Pythium* spp. are ubiquitous in greenhouse environments and rapidly increase and spread in hydroponic systems (26). Such practices include disinfestation of the nutrient solution as it recirculates outside the crop using ultraviolet (UV) irradiation, heat pasteurization, filtration, and ultrasonic treatment (11,25,26). In our feasibility tests of hydroponic chrysanthemum, UV treatment of recirculating nutrient solution at doses sufficient to kill or inactivate *Pythium* propagules (27) failed to suppress progress of root rot. Similarly, UV treatment did not significantly reduce progress of *Pythium* root rot in tomatoes, lettuce, chrysanthemum, or sweet pepper in small-scale hydroponic systems (29) or in a commercial pepper crop (22). The ineffectiveness of disinfestation as a means to control root rot probably relates to low frequency of zoospore dispersal in nutrient solution recirculating outside the crop (26) and emphasizes the need to control *Pythium* in the root zone.

Several investigators reported that specific strains of bacteria and fungi introduced into the root zone effectively suppressed *Pythium* root rot in greenhouse vegetables and flowers grown in small-scale hydroponic systems (3,7,13,15,30). While these investigations were chiefly short-term (*e.g.* 12–35 days), longer term (*e.g.* 2–6 mo) control also was demonstrated in greenhouse vegetables produced in small- and commercial-scale hydroponic systems (8,24,30). These findings, and those in a preliminary study in chrysanthemum (9), suggested that antagonistic microbes justify further investigation as tools for root-zone protection of hydroponic chrysanthemums.

It has long been recognized that the effectiveness of disease control measures is best understood as a function of environmental conditions and human interference (21). Thus, when Pythium root rot and its biological control are investigated in small-scale hydroponic systems, it is desirable that conditions of the root and aerial environments be representative of those in the commercial crop. In Ontario greenhouses, conditions of the hydroponic nutrient solution and canopy microclimate are in most instances controlled

within prescribed limits. However, temperature in the root zone is sometimes sufficiently high (27–35°C) to strongly favor epidemics of Pythium root rot in various crops (26), and may do so also in chrysanthemum. High temperature, and other conditions conducive to disease, can be expected to influence the effectiveness of biological control.

The present investigations evaluated root-colonizing bacteria to control root rot caused by *P. aphanidermatum* or *P. dissotocum* in hydroponic chrysanthemums. The plants were grown in single-plant hydroponic units to allow versatility in the number of treatments evaluated, adequate treatment replication, repeated observations of root systems, and mobility of plants such as for inoculations and for transfer to temperature-controlled water baths. Effects of nutrient solution temperature on disease progress were quantified and used to set conditions in biological control experiments. A range of bacterial strains, including several reported to suppress Pythium root rot in other crops, were tested against the pathogens in chrysanthemum roots and on an agar medium. Effects of inoculum density and timing of bacterial treatments were quantified in chrysanthemum plants kept at different temperatures to provide a basis for microbial control of Pythium root rot.

MATERIALS AND METHODS

Plant materials and hydroponic units Plants of chrysanthemum cv. 'Fina' were grown from cuttings rooted in rockwool plugs (2.5 cm × 2.5 cm × 4.0 cm) (Grodan, Roermond, The Netherlands) in plastic trays in a research greenhouse and transferred to single-plant hydroponic units when 8–10 cm tall. In each unit, a plant was positioned with the plug supported in a square hole (2.5 cm × 2.5 cm) in the center of the lid of a 500-ml or 1.9-*l* white plastic container that was filled with nutrient solution. To exclude light, the container was inserted into a black plastic pot, and the lid and plug were covered with black-on-white plastic sheeting, black side downwards. The nutrient solution was prepared with 0.73 g of soluble fertilizer (N:P:K, 7:11:27; Plant Products Ltd., Brampton, ON, Canada) and 0.48 g $Ca(NO_3)_2$ per liter of water and adjusted to pH 5.5–5.8 with HNO_3. Electrical conductivity (EC) of the solution was 1.6–1.8 $mS{\cdot}cm^{-1}$. For aeration, compressed air with flow regulated by an aquarium air valve was bubbled continuously into the solution in each container *via* plastic tubing (2 mm i.d.). The hydroponic units were positioned on the greenhouse bench such that the chrysanthemum stems were 30–35 cm apart. Nutrient solution lost by transpiration and evaporation was replenished daily. The pH, EC, and dissolved oxygen content (DOC) of the solution were measured every 3 to 4 days and adjusted as required. DOC was measured with an oxygen meter (model YSI 55, Yellow Springs Instruments Co. Inc., Yellow Springs, OH, USA). Solution temperature was measured in four arbitrary units at approximately 0900, 1400 and 2000 hours EST on alternate days by means of a portable pH and temperature meter (Accumet model AP61, Fisher Scientific, Toronto, Canada). Hourly air temperature values, measured by thermistors near the center of the greenhouse unit, were obtained from computer records of the greenhouse complex.

Inoculum production and inoculations *Pythium aphanidermatum* isolate P6 from hydroponic cucumber and *P. dissotocum* isolate P15 were used for inoculations (12). Zoospores of the isolates for inoculations were produced by a method modified from Rahimian and Banihashemi (17) and fully described by Owen-Going *et al.* (12). In summary, the isolates were cultured on 10% V-8 juice agar medium in petri dishes and the colonies were flooded with sterile water after 48 h of incubation and again after 96 h in a

critically controlled temperature regime. To estimate density of zoospores freshly released into the water, 1-ml aliquots of zoospore suspension were vortexed in microcentrifuge tubes for 1 min and the immobilized zoospores were counted on a hemocytometer. Suspensions were diluted to 1×10^4 zoospores ml^{-1} with half-strength plant nutrient solution for use in inoculations. To inoculate each plant, roots extending below the rockwool plug were immersed in 60-ml zoospore suspension for 30 min and the plant was immediately returned to the hydroponic unit.

The following bacterial strains were used in the experiments: *Pseudomonas fluorescens* 15 (T.C. Paulitz, McGill University, Montreal, QC, Canada); *Ps. fluorescens* JZ12 (J. Zheng, Agriculture and Agri-Food Canada [AAFC], Harrow, Ont., Canada); *Ps. fluorescens* HY05 and *Bacillus cereus* HY06 (H. Yu, University of Guelph, Canada); *Pseudomonas chlororaphis* Tx-1 (= *Ps. aureofaciens* Tx-1) and *Ps. chlororaphis* 63-28 (Turf Science Laboratories Inc., National City, CA, USA); *Pseudomonas corrugata* 13 (J. Gracia-Garza, AAFC, Vineland, Ont., Canada); *Burkholderia gladioli* C-2-74 and *Comamonas acidovorans* C-4-7-12, C-4-7-22, C-4-7-28, OCR-7-8-39 and OCR-7-8-38: J.W. Kloepper). The bacteria were grown as shake cultures in tryptic soy broth at 21–23°C for 24 h, recovered by centrifugation, and suspended in 0.1 M $MgSO_4$. Density of colony-forming units (CFU) in cell suspensions of each strain was estimated from absorbance measurements at 640 nm and a standard curve for absorbance and CFU ml^{-1}. For treating plants, bacteria were introduced into the nutrient solution at final densities specified in descriptions of experiments given below.

Assessment of disease and colonization Percent total roots that were discolored was estimated visually using an equal-increment scale of 0–10 (0 = 0%, 1 = 1–10%, 2 = 11–20%.... 10 = 91–100%). Discoloration was generally grayish-brown or reddish-brown. For each estimation, each plant was held with the roots above the nutrient solution and examined for about 15–20 s. Mid-point values of scale increments were used for data analysis.

To estimate percent roots colonized by *Pythium*, 20 principal roots were removed from each plant and cut into 1-cm-long segments. The segments were surface-disinfested in 1% NaOCl for 30 s and in 70% ethanol for 30 s, rinsed three times in sterile distilled water and blotted dry. Thirty random segments from each plant were placed on the P_5ARP selective medium of Jeffers and Martin (4). Incidence of segments with *Pythium* colonies was recorded after 48 h at 25°C.

Effects of temperature on disease progress Plants for investigation of temperature in relation to Pythium root rot were grown in 1.9-*l* single-plant hydroponic units in a research greenhouse at approximately 25°C (day) and 22°C (night), and with the photoperiod extended to 16 h by means of sodium vapor lamps. When 15–18 cm tall, the plants were inoculated with *P. aphanidermatum* or *P. dissotocum* and moved into four Plexiglas®temperature chambers, each 106 cm × 76 cm × 90 cm high, positioned on a bench beneath a bank of cool-white fluorescent lamps in a growth room maintained at 20°C. Air temperature in the respective chambers was maintained at 20±0.5°, 24±0.5°, 28± 0.5° and 32±0.5°C by means of thermostatically controlled fan heaters. After equilibration during approximately 6 h, temperature of the nutrient solution ranged up to 1.5°C below the air temperature. Root disease was estimated daily for 18 days after inoculation. There were six plants per treatment, and the experiment was conducted twice.

Antibiosis assays The bacteria were evaluated for antibiosis against *P. aphanidermatum* and *P. dissotocum* in assays conducted in 9-cm-diam petri dishes containing V-8 juice agar medium covered by cellophane membrane (pore size 0.22 μm; Fisher Scientific). A cell suspension (0.1 ml) of each agent (10^7 CFU ml^{-1} of 0.1 M $MgSO_4$) or 0.1 M $MgSO_4$ only was spread on the entire membrane in each of a series of petri dishes. After incubation at 21–23°C for 14 days, the membranes were peeled off and a 0.5-cm-diam plug from a 2-day-old colony of *P. aphanidermatum* or *P. dissotocum* on V-8 juice agar medium was positioned at the center of each dish where the membrane had been. The diameter of the *Pythium* colony in each disk was measured after 6, 12, 24, 48 and 60 h at 21–23°C. There were four replicate dishes per treatment, and the experiment was conducted twice.

Effectiveness of bacteria against *Pythium* in chrysanthemum roots The bacteria were evaluated for effectiveness in suppressing symptom development in roots of chrysanthemum plants inoculated with *P. aphanidermatum* or with *P. dissotocum* in the research greenhouse. Each strain was introduced into the nutrient solution at a final density of 10^4 CFU ml^{-1} solution 7 days after the plants were positioned in 1.9-*l* hydroponic units. Plants were inoculated with *P. aphanidermatum* or *P. dissotocum* after a further 14 days. Control plants received no agent and no *Pythium* (untreated control), or one of the *Pythium* spp. but no agent (pathogen controls). The percent of roots discolored was estimated immediately before and at 2-day intervals after inoculation until day 18, when colonization of roots by *P. aphanidermatum* or *P. dissotocum* was estimated. There were four plants per treatment in each of two repetitions of the experiment.

Five of the bacteria (*Ps. fluorescens* 15, *B. cereus* HY06, *B. gladioli* C-2-74, *Ps. chlororaphis* Tx-1 and *Ps. chlororaphis* 63-28) were investigated to determine whether a tenfold reduction in density of applied CFU would influence their effectiveness against *P. aphanidermatum* and *P. dissotocum*. The bacteria were introduced at final densities of 10^3 CFU and 10^4 CFU ml^{-1} nutrient solution, and roots were inoculated with *P. aphanidermatum* or *P. dissotocum* 14 days later. Root discoloration was estimated immediately before and every 1 to 2 days after inoculation, and colonization of roots by the pathogens was assessed on day 12. The experiment was otherwise the same as the preceding experiment, and was conducted twice.

To examine relationships between treatment timing and agent effectiveness, four bacteria (*Ps. fluorescens*, *B. gladioli*, *Ps. chlororaphis* Tx-1 and *Ps. chlororaphis* 63-28) were applied at a final density of 10^4 CFU ml^{-1} nutrient solution at 14, 7 and 0 days before the roots were inoculated with *P. aphanidermatum*. Root discoloration was assessed as before, and colonization was estimated on day 14. All other conditions were the same as described for the preceding biocontrol studies, and the experiment was conducted twice.

Effects of temperature on biological control Root zone temperature was examined in relation to effectiveness of *B. cereus*, *B. gladioli*, *Ps. chlororaphis* Tx-1 and *Ps. chlororaphis* 63-28 against *P. aphanidermatum* in chrysanthemum plants maintained in 500 ml hydroponic units. The units were positioned in hydroponic plant trays (100 cm × 38 cm × 19 cm deep; Homegrown Hydroponics Inc., Burlington, Ont., Canada), made entirely of black plastic, which served as controlled-temperature water baths. A cover mounted inside the top of the tray supported up to twenty of the hydroponic units in 9-cm-diam holes spaced equidistantly in three lengthwise rows. Water was maintained to the level of the cover in each tray and heated by means of a thermostatically controlled heating cable

that was looped in rows ∼6 cm apart on galvanized wire mesh 1 cm above the base of the tray. The trays were placed on a bench beneath a bank of cool-white fluorescent lamps in a growth room maintained with a 16-h photoperiod at 22±1°C. Plants in rockwool plugs were transferred to the hydroponic units in the trays when 10–12 cm tall. Irradiance at plant height was 190–200 μmol m^{-2} s^{-1}. Water temperature was regulated so as to maintain the temperature of the nutrient solution near 22°C for 7 days and subsequently equilibrated at 24±0.5° or 32±0.5°C. The bacterial strains were then introduced into the nutrient solution at a final density of 10^4 CFU ml^{-1}. Plants were inoculated with *P. aphanidermatum* 14 days after bacterial treatments.. Control plants received no bacteria and no *Pythium* (untreated control), or *Pythium* but no bacteria (pathogen control). Discoloration of roots was estimated immediately before inoculation and at 2-day intervals thereafter. At 14 days after inoculation, roots exterior to the rockwool plugs were removed and used to estimate percent colonization by the pathogen and total dry mass. There were four replicate plants per treatment. The experiment was designed as a randomized complete block and conducted twice.

Effects of treatment timing on biological control The strains of *B. cereus*, *B. gladioli*, *Ps. chlororaphis* Tx-1 and *Ps. chlororaphis* 63-28 were introduced into the nutrient solution at a final density of 10^4 CFU ml^{-1} solution at 14 or 7 days before, or 1 h before, plants were inoculated with *P. aphanidermatum*. The study was conducted in the research greenhouse using 1.9-*l* hydroponic units. Control plants received no agent and no *Pythium* (untreated control), or *Pythium* only (pathogen control). The percent roots discolored was estimated immediately before and at 1- to 2-day intervals after inoculation, and colonization of roots by *P. aphanidermatum* at day 14. There were four plants per treatment in each of two repetitions of the experiment.

Statistical analysis Data for percent discolored roots were used to estimate values for area under the discoloration progress curve (AUDPC) for each treatment according to the equation:

$$AUDPC = \sum_{i}^{n-1}((y_i + y_i + 1)/2)(t_i + 1 - t_i)$$

in which *n*, number of days when disease was assessed; y_i, percent roots discolored on the *i*th day; and t_i, *i*th day (19). AUDPC values were calculated for the duration of each experiment beginning on the day of pathogen inoculation. Variance analysis was conducted to determine the significance of treatment factors on the AUDPC values using the Statistical Analysis System, version 8 (18). Variance analysis also was performed for data of root discoloration, root colonization by *Pythium*, and dry mass of roots obtained on the final day of each experiment in the hydroponic units, and for data of colony diameter of the *Pythium* spp. in the petri dish assays. Means of AUDPC values and of the data for root discoloration, root colonization and root dry mass, from plants treated with bacteria and inoculated with *P. aphanidermatum* or *P. dissotocum* were compared using T-test grouping LSD (1). Relationships between root discoloration and root colonization were examined by simple correlation analysis and Spearman's rank correlation. Means of AUDPC values for root discoloration as a function of temperature, and of observations of *Pythium* colony diameter in petri dish assays, were compared using T-test grouping LSD.

Experiments on effectiveness of bacteria against *Pythium*, and on effects of temperature

TABLE 1. Effects of nutrient solution temperature on AUDPC values for discolored roots in hydroponic chrysanthemum plants at 0–18 days after inoculation with *Pythium aphanidermatum* or *Pythium dissotocum*

	AUDPC values	
Temperature (°C)	*P. aphanidermatum*	*P. dissotocum*
20	119 a[z]	119 a
24	240 ab	240 b
28	389 b	240 b
32	690 c	330 c

[z] Within columns, values followed by a common letter do not differ significantly (T-test grouping LSD, $P<0.05$).

and of bacterial treatments on biological control, were subjected to factorial analysis to determine effects and interactions of treatments on AUDPC values, root discoloration, root colonization and root dry mass.

Normality of all dependent variables was tested by means of the univariate procedure, and homogeneity of experimental errors was examined by plotting residuals *vs* predicted values using the mixed model and plot procedure (1). Data for repetitions of the experiments were pooled when *F*-tests indicated that variances of the data did not differ significantly. The Type 1 error rate ($\propto$) was set at 0.05 for all statistical tests.

RESULTS

Effect of temperature on disease progress Observations of the two experimental repetitions did not differ significantly ($P>0.01$) and were pooled. During 18 days after plants were inoculated with *P. aphanidermatum,* root discoloration increased to 14%, 27%, 43% and 77%, respectively, at 20°, 24°, 28° and 32°C (Fig. 1). Discoloration at each temperature progressed mainly in a single step that began at 3 days (24–32°C) or 7 days (20°C) after inoculation and ended at 7–9 days. Thereafter discoloration plateaued or increased only slowly at levels that were about six times higher at 32° than at 20°C. Greatest severity of root discoloration associated with *P. dissotocum* was 14%, 27%, 27% and 37%, respectively, at 20°, 24°, 28° and 32°C (Fig. 1). Progress of root discoloration in plants inoculated with *P. dissotocum* was similar to that caused by *P. aphanidermatum* at 20° and 24°C, but plateaued at levels 40% and 52% lower, respectively, at 28° and 32°C. AUDPC values for *P. aphanidermatum* were 5.8 and 3.3 times higher at 32° and 28°C, respectively, than at 20°C, whereas AUDPC values for *P. dissotocum* were only 2.8, 2.0 and 2.0 times higher, respectively, at 32°, 28° and 24°C compared with 20°C (Table 1).

Antibiosis by bacteria In the antibiosis assays, variance of observations of colony growth did not differ significantly and therefore the data were combined for analysis. Colonies of *P. aphanidermatum* and *P. dissotocum* in the untreated controls grew to the edge of the agar medium within 36 h and 48 h, respectively. At 36 h the bacterial strains significantly suppressed growth of *P. aphanidermatum* in the range of 33–92% (Table 2). *Ps. fluorescens* 15, *Ps. fluorescens* HY05 and all strains of *C. acidovorans* were mildly suppressive (33–60%); *Ps. fluorescens* JZ12, *Ps. corrugata* 13 and *B. gladioli* C-2-74 were moderately suppressive (71–82%); and *Ps. chlororaphis* Tx-1, *Ps. chlororaphis* 63-28 and *B. cereus* HY06 were strongly suppressive (90–92%). Observations at 60 h indicated that *Ps. chlororaphis* Tx-1 and 63-28, *B. cereus* HY06, *Ps. fluorescens* JZ12 and *B. gladioli* C-2-74 maintained strong suppression (78–91%); *Ps. fluorescens*, *Ps. fluorescens* HY05

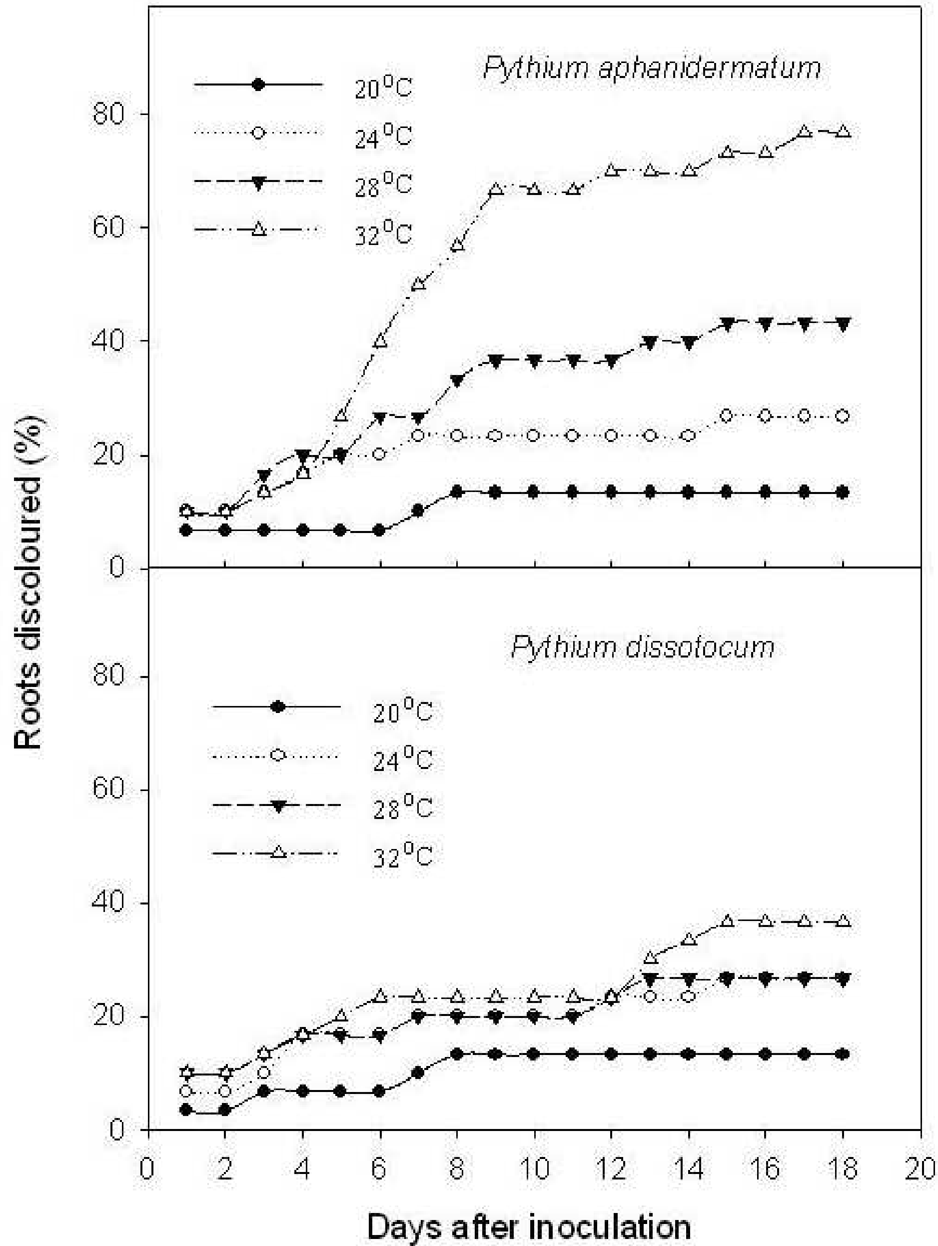

Fig. 1. Relationship of temperature to root discoloration caused by *Pythium aphanidermatum* and *Pythium dissotocum* in chrysanthemum plants grown in single-plant hydroponic units.

and *Ps. corrugate* moderate suppression (50–66%); and the isolates of *C. acidovorans* little or no suppression (0–18%).

Observations at 36 h indicated that all of the bacteria also suppressed colony growth

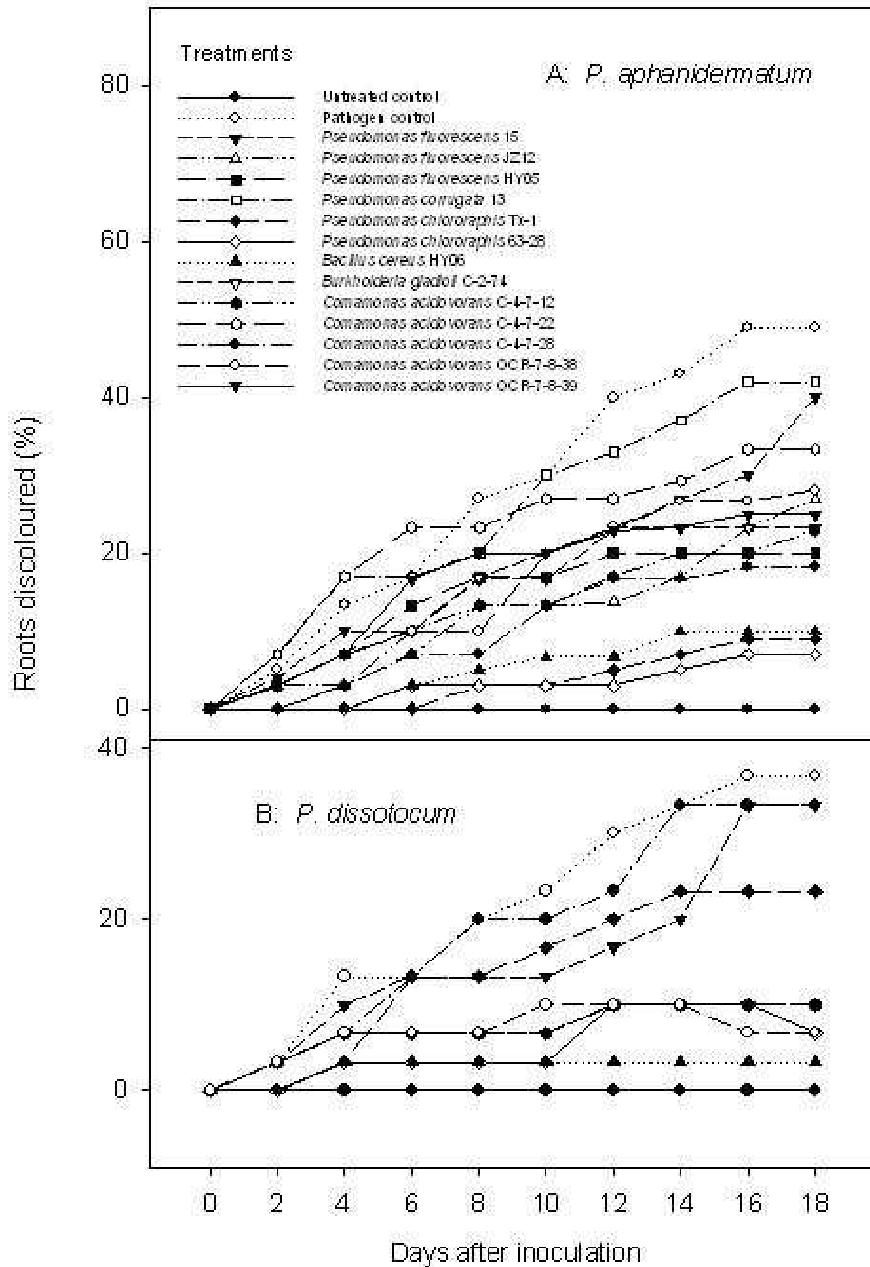

Fig. 2. Effects of bacteria on progress curves for percent roots discolored in hydroponic chrysanthemum plants inoculated with *Pythium aphanidermatum* or *Pythium dissotocum* 14 days after the bacteria were introduced into the nutrient solution.

of *P. dissotocum* (Table 2). *Ps. fluorescens* 15, *Ps. fluorescens* HY05 and all strains of *C. acidovorans* were mildly suppressive (15–47%) to *P. dissotocum*, as was found for

TABLE 2. Influence of microbial agents on colony growth of *Pythium aphanidermatum* and *Pythium dissotocum* estimated in a cellulose membrane assay on V8 juice agar medium

Microbial agent	Colony diameter (cm)			
	P. aphanidermatum		*P. dissotocum*	
	36 h	60 h	36 h	60 h
None	8.9 a[z]	9.0 a	5.9 a	9.0 a
Pseudomonas fluorescens 15	3.6 f	5.1 e	3.6 e	5.0 c
Pseudomonas fluorescens JZ12	1.6 h	2.0 g	1.1 g	1.2 h
Pseudomonas fluorescens HY05	4.3 de	5.5 d	3.9 d	4.2 e
Pseudomonas corrugata 13	2.6 g	3.0 f	1.2 g	1.5 gh
Pseudomonas chlororaphis Tx-1	0.9 i	1.1 hi	3.8 d	4.3 de
Pseudomonas chlororaphis 63-28	0.7 i	0.8 i	0.8 h	1.1 h
*Bacillus cereus*HY06	0.8 i	1.4 h	1.4 g	1.7 g
Burkholderia gladioli C-2-74	1.6 h	1.9 g	0.6 h	1.7 g
Comamonas acidovorans C-4-7-12	4.3 de	8.2 b	4.4 c	5.0 c
Comamonas acidovorans C-4-7-22	6.0 b	9.0 a	5.0 b	8.8 b
Comamonas acidovorans C-4-7-28	4.1 e	8.5 b	3.1 f	3.7 f
Comamonas acidovorans OCR-7-8-38	4.6 cd	9.0 a	4.0 cd	9.0 a
Comamonas acidovorans OCR-7-8-39	4.8 c	7.4 c	3.9 d	4.5 d

[z] Within columns, values followed by a common letter do not differ significantly (T-test grouping LSD, $P<0.05$).

P. aphanidermatum, but *Ps. chlororaphis* Tx-1 also was only mildly suppressive (36%) against *P. dissotocum*. Strains of *Ps. fluorescens* JZ12, *Ps. corrugata* 13, *Ps. chlororaphis* 63-28, *B. cereus* HY06 and *B. gladioli* C-2-74 were strongly suppressive at 36 h (76–90%) and also at 60 h (81–88%).

Effectiveness of bacteria against *Pythium* in chrysanthemum roots Variance of observations in the two repetitions of the biological control studies against *P. aphanidermatum* and *P. dissotocum* differed significantly (P=0.05), but the data were similar in pattern and correlated strongly (for *P. aphanidermatum*, r = 0.93; $P<0.001$; for *P. dissotocum*, r = 0.89; P=0.05). Data of the repetition with greatest disease severity are reported. No root discoloration was observed in the untreated controls. In the study with *P. aphanidermatum*, root discoloration in the pathogen control increased to 49% during 16 days after inoculation (Fig. 2A). Progress curves of root discoloration were generally lower than in the pathogen control when plants were treated with the bacterial strains 14 days before the roots were inoculated with the pathogen. *Ps. fluorescens* HY05, *Ps. chlororaphis* Tx-1, *Ps. chlororaphis* 63-28, *B. cereus* HY06, *B. gladioli* C-2-74 and *C. acidovorans* C-4-7-12, C-4-7-28 and OCR-7-8-39 significantly reduced AUDPC values by 42–66% when compared with the *P. aphanidermatum* controls (Table 3). These strains in most instances delayed initial root discoloration by 4–8 days (Fig. 2A), and significantly reduced percent root colonization associated with *P. aphanidermatum* by 54–84% and percent roots discolored by 49–86% on day 18 (Table 3). The strains *B. cereus* HY06, *Ps. chlororaphis* Tx-1 and *Ps. chlororaphis* 63-28 controlled discoloration below 10% during the 18 days after the plants were inoculated with *P. aphanidermatum*. Strong correlations and rank correlations were obtained for bacterial effectiveness against colonization and discoloration of roots by *P. aphanidermatum* (Table 3). The AUDPC values correlated only weakly with percent suppression of colony growth of *P. aphanidermatum* by various strains in the antibiosis assays (r=0.41, $P<0.05$).

In the study with *P. dissotocum*, progress curves for root discoloration in plants treated with the bacterial strains were generally lower than the curve for the pathogen control

TABLE 3. Effects of microbial agents applied to hydroponic chrysanthemum plants 14 days before inoculation with *Pythium aphanidermatum* (*Pa*) or *Pythium dissotocum* (*Pd*) on AUDPC values for discolored roots during 0–18 days after inoculation, and on root colonization by the pathogen and root discoloration estimated on day 18

Treatments	*P. aphanidermatum*					*P. dissotocum*				
		Colonization		Discoloration			Colonization		Discoloration	
	AUDPC	(%)	Rank[z]	(%)	Rank	AUDPC	(%)	Rank	(%)	Rank
None	0	0			0	0	0	0		
Pathogen control	387 a[y]	90 a		49 a		302 a	77 a		37 a	
Pseudomonas fluorescens 15+Pathogen 360 a	67 ab	12		40 a	12	299 a	57 ab	7	34 a	7
Pseudomonas fluorescens JZ12+Pathogen	243 abc 39 cd 7	27 ab 9	NT[x]							
Pseudomonas fluorescens HY05+Pathogen	180 cd	41 bc	9	20 bc	5	NT				
Pseudomonas corrugata 13+Pathogen	374 a	72 ab	13	41 a	13	NT				
Pseudomonas chlororaphis Tx-1+ Pathogen	133 d	14 d	1	9 c	2	210 ab	17 c	4	23 ab	5
Pseudomonas chlororaphis 63-28+Pathogen	150 d	15 d	2	7 c	1	90 cd	10 c	2	7 c	2
Bacillus cereus HY06+Pathogen	135 d	25 cd	4	10 c	3	30 d	7 c	1	3 c	1
Burkholderia gladioli C-2-74+Pathogen	210 cd	26 cd	5	23 b	6	120 bc	20 bc	5	10 bc	4
Comamonas acidovorans C-4-7-12+Pathogen	207 cd	66 abc	11	23 b	7	NT				
Comamonas acidovorans C-4-7-22+Pathogen	300 ab	65 abc	10	33 ab	11	NT				
Comamonas acidovorans C-4-7-28+Pathogen	165 cd	18 cd	3	18 bc	4	300 a	40 b	6	33 a	6
Comamonas acidovorans OCR-7-8-38+Pathogen	252 abc	39 cd	6	28 ab	10	90 cd	11 c	3	8 c	3
Comamonas acidovorans OCR-7-8-39+Pathogen	225 bcd	39 cd	8	25 b	8	NT				

Correlation coefficient for % colonization *vs* % discoloration: *Pa*: r = 0.93 $P<0.001$ *Pd*: r = 0.92 $P<0.001$

Rank correlation coefficient for % colonization *vs* % discoloration: *Pa*: r = 0.84 $P<0.0003$ *Pd:* r = 0.97 $P<0.0001$

[z] Treatments ranked for effectiveness based on mean values for colonization or discoloration.

[y] Within columns, means followed by a common letter do not differ significantly (T-test grouping LSD, $P<0.05$).

[x] NT: not tested.

TABLE 4. Effects of reduced inoculum density of bacterial strains, applied to chrysanthemum plants 14 days before inoculation with *Pythium aphanidermatum* (*Pa*) or *Pythium dissotocum* (*Pd*), on AUDPC values for discolored roots, and on colonization of the roots and root discoloration estimated at 18 days after inoculation

Treatments	*P. aphanidermatum*			*P. dissotocum*		
	AUDPC	Colonization (%)	Discoloration (%)	AUDPC	Colonization (%)	Discoloration (%)
None	0	0	0	0	0	0
Pathogen control	630 a[y]	100 a	59 a	270 a	77 a	30 a
10^3 CFU ml^{-1}[z]						
Pseudomonas fluorescens 15+Pathogen	360 bcd	93 a	43 ab	240 ab	57 abc	27 a
Pseudomonas chlororaphis Tx-1+Pathogen	240 cde	27 c	27 bc	210 ab	63 ab	23 ab
Pseudomonas chlororaphis 63-28+Pathogen	210 de	32 bc	30 bc	90 cd	23 c	10 c
Bacillus cereus HY06+Pathogen	240 cde	20 c	23 bc	120 bc	40 abc	13 bc
Burkholderia gladioli C-2-74+Pathogen	480 ab	68 ab	57 a	180 abc	63 ab	20 b
10^4 CFU ml^{-1}[z]						
Pseudomonas fluorescens 15+Pathogen	180 de	33 bc	20 c	240 ab	66 a	27 a
Pseudomonas chlororaphis Tx-1+Pathogen	120 e	25 c	23 bc	150 abc	57 abc	23 ab
Pseudomonas chlororaphis 63-28+Pathogen	150 e	33 bc	23 bc	60 d	27 bc	10 c
Bacillus cereus HY06+Pathogen	180 de	17 c	17 c	60 d	50 abc	13 bc
Burkholderia gladioli C-2-74+Pathogen	430 abc	37 bc	33 abc	120 bc	30 bc	13 bc

Correlation coefficient for % colonization *vs* % discoloration for *Pa*: r = 0.92, $P<0.0001$; for *Pd*: r = 0.88, $P<0.01$.

[z] Final inoculum density of agents in CFU ml^{-1} nutrient solution.

[y] Within columns, means followed by a common letter do not differ significantly (T-test grouping LSD, $P<0.05$).

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TABLE 5. Influence of nutrient solution temperature (24° or 32°C) on effectiveness of bacterial strains against discoloration and pathogen colonization in roots of hydroponic chrysanthemum plants inoculated with *Pythium aphanidermatum* (*Pa*) 14 days after treatment with the agents

	AUDPC[z]	Suppression of AUDPC[y] (%)	Colonization (%)	Discoloration (%)	Dry mass of roots (%)
24°C					
None	0	–	0	0	7.7 a
Pa control	117 a[x]	–	77 a	17 a	4.8 b
Pseudomonas chlororaphis Tx-1 + *Pa*	23 c	80	30 b	3 b	6.7 ab
Pseudomonas chlororaphis 63-28 + *Pa*	47 bc	63	33 b	7 b	5.4 b
Bacillus cereus HY06 + *Pa*	70 ab	40	33 b	10 ab	7.4 a
Burkholderia gladioli C-2-74 + *Pa*	93 ab	21	50 ab	13 a	5.4 b
32°C					
None	0	–	0	0	1.6 b
Pa control	373 a	–	100 a	53 a	1.5 bc
Pseudomonas chlororaphis Tx-1 + *Pa*	163 b	56	47 b	23 b	2.4 a
Pseudomonas chlororaphis 63-28 + *Pa*	187 b	50	53 b	27 b	1.9 b
Bacillus cereus HY06 + *Pa*	210 b	44	53 b	30 b	1.6 b
Burkholderia gladioli C-2-74 + *Pa*	327 a	12	83 a	47 a	1.3 c

Correlation coefficient: % colonization *vs* % discoloration: $r = 0.84$ $P<0.01$

[z] Area under the disease progress curve for 0 to 14 days after roots were inoculated with *Pa*.

[y] Suppression of AUDPC by bacterial strains compared with *Pa* control.

[x] Within columns, and for a given temperature, means followed by a common letter do not differ significantly (T-test grouping LSD, $P<0.05$).

TABLE 6. Effects of application of bacterial strains to the nutrient solution of hydroponic chrysanthemum plants at 14, 7 or 0 days before the roots were inoculated with *Pythium aphanidermatum (Pa)*, on AUDPC values for discolored roots during 14 days after inoculation, and on colonization of the roots by the pathogen and percent discolored roots estimated on day 14 after inoculation

Treatments	AUDPC	Colonization of roots		Discoloration of roots	
		(%)	Rank[z]	(%)	Rank
None	0	0		0	
Pa control	373 a[y]	100 a		45 a	
Pseudomonas chlororaphis Tx-1 (14)[x] + *Pa*	47 d	30 c	3	13 c	3
Pseudomonas chlororaphis 63-28 (14) +*Pa*	23 d	37 c	4	17 c	5
Bacillus cereus HY06 (14) + *Pa*	93 cd	13 c	1	10 c	1
Burkholderia gladioli C-2-74 (14) + *Pa*	93 cd	23 c	2	20 bc	6
Pseudomonas chlororaphis Tx-1 (7) + *Pa*	46 d	56 bc	6	10 c	2
Pseudomonas chlororaphis 63-28 (7) + *Pa*	70 d	53 bc	5	13 c	4
Bacillus cereus HY06 (7) + *Pa*	186 bc	60 abc	7	33 ab	10
Burkholderia gladioli C-2-74 (7) + *Pa*	256 ab	60 abc	8	40 a	11
Pseudomonas chlororaphis Tx-1 (0) + *Pa*	140 bc	76 ab	11	27 bc	9
Pseudomonas chlororaphis 63-28 (0) + *Pa*	140 bc	69 abc	9	23 bc	7
Bacillus cereus HY06 (0) + *Pa*	186 bc	86 ab	12		
Burkholderia gladioli C-2-74 (0) + *Pa*	286 ab	71 ab	10	43 a	12

Correlation coefficients: % colonization *vs* % discoloration:r = 0.81 $P<0.005$ colonization rank *vs* discoloration rank: r = 0.86 $P<0.01$

[z]Treatments ranked for effectiveness based on mean values for colonization or discoloration.

[y]Within columns, means followed by a common letter do not differ significantly (T-test grouping LSD, $P<0.05$).

[x]Time of application of bacterial strains in days (7 or 14) before roots were inoculated with *P. aphanidermatum*.

(Fig. 2B), but only *Ps. chlororaphis* 63-28, *B. cereus* HY06, *B. gladioli* C-2-74 and *C. acidovorans* OCR-7-8-38 significantly reduced AUDPC values (reductions 60–90%; Table 3). These strains also reduced root colonization by *P. dissotocum* by 74–91% and root discoloration by 73–92% estimated on day 18. At that time, *Ps. chlororaphis* Tx-1 and *C. acidovorans* C-4-7-28 significantly reduced colonization by the pathogen but not root discoloration. Strong correlations and rank correlations were found for effectiveness of the bacterial strains against colonization and root discoloration in plants inoculated with *P. dissotocum* (Table 3). The AUDPC values correlated with percent suppression of colony growth of *P. dissotocum* in the antibiosis assays (r=0.63, $P<0.05$).

In plants inoculated with *P. aphanidermatum*, the bacterial strains *Ps. chlororaphis* Tx-1, *Ps. chlororaphis* 63-28 and *B. cereus* HY06 variously reduced AUDPC values by 62–67%, colonization by 68–80% and discoloration by 49–61% when applied in the nutrient solution at 10^3 CFU ml^{-1} (Table 4). Respective values when applied at 10^4 CFU ml^{-1} were 71–81%, 67–83% and 61–71%. *Ps. fluorescens* 15 suppressed the value of each variable when introduced at 10^4 CFU ml^{-1} but reduced only AUDPC (by 43%) when applied at 10^3 CFU ml^{-1}. *B. gladioli* C-2-74 was generally ineffective. Values for percent colonization and percent discoloration of roots among the treatments correlated strongly (Table 4). The ANOVA indicated that there was no significant interaction (P=0.3512) between effects of the bacterial strains at each concentration.

In plants inoculated with *P. dissotocum*, *Ps. chlororaphis* 63-28 and *B. cereus* HY06 significantly reduced AUDPC values by 67–78% and 56–78%, respectively, when applied at 10^3 or 10^4 CFU ml^{-1}, and *B. gladioli* C-2-74 did so by 56% when introduced at 10^4 CFU ml^{-1} (Table 4). The most consistent performance in terms of suppressing values for AUDPC, colonization, and root discoloration was obtained with *Ps. chlororaphis* 63-28. *Ps. fluorescens* 15 and *Ps. chlororaphis* Tx-1 were ineffective against *P. dissotocum* regardless of inoculum density employed.

Effects of temperature on biological control Variances of observations in the two experimental repetitions did not differ significantly, so the data were combined for analysis. No root discoloration or colonization of roots by *P. aphanidermatum* was observed in untreated control plants maintained in nutrient solution at 24° or 32°C, but mean dry mass of the roots at 14 days after plants were inoculated was more than four times lower at 32° than at 24°C (Table 5). In the pathogen controls, values for AUDPC and for percent root discoloration on day 14 were approximately three times greater at 32° than at 24°C, while the pathogen was recovered from 100% of root segments at 32° and 77% at 24°C. Compared with the pathogen controls at 24° and 32°C, respectively, *Ps. chlororaphis* Tx-1 suppressed AUDPC by 80% and 56%, root discoloration by 82% and 57%, and colonization by 61% and 57%; *Ps. chlororaphis* 63-28 reduced AUDPC by 63% and 50%, root discoloration by 59% and 57%, and colonization by 57% and 47%. *Bacillus cereus* HY06 did not significantly reduce AUDPC at 24° but reduced it by 44% at 32°C; at 24° and 32°C, respectively, this strain reduced root discoloration by 41% and 43%, and colonization by 57% and 47%. Compared with the pathogen controls, *Ps. chlororaphis* Tx-1 increased root dry mass by 60% at 32° but had no significant effect at 24°, whereas *B. cereus* HY06 increased root dry mass by 54% at 32° but had no effect at 24°C. *B. gladioli* C-2-74 did not significantly affect any of the dependent variables.

Effects of treatment timing on biological control Variances of observations of the two experimental repetitions did not differ significantly, and therefore the data were combined for analysis. In the ANOVA, no interaction was found between effects of the bacterial strains and time of application. All strains significantly suppressed AUDPC values in the range of 23–94% compared with the pathogen control when applied to the nutrient solution at 14, 7 or 0 days (1 h) before plants were inoculated with *P. aphanidermatum*, except for *B. gladioli* C-2-74 – which was ineffective when applied at 7 or 0 days (Table 6). Similarly, application of the strains at 14, 7 or 0 days before inoculation significantly reduced root discoloration in the range of 40–78% at 14 days after inoculation, except for *B. cereus* HY06 applied at 7 days and *B. gladioli* applied at 7 or 0 days before inoculation. Root colonization by *P. aphanidermatum* estimated at 14 days after inoculation was reduced by 70–87% when the various strains were applied at 14 days before inoculation, but only by *Ps. chlororaphis* Tx-1 and *Ps. chlororaphis* 63-28 when applied at 7 days. No strain reduced colonization when applied 1 h before inoculation. A strong correlation (r=0.81) was found between percent roots colonized and percent roots discolored among the treatments at 14 days after inoculation.

DISCUSSION

The observations showed that several bacterial strains reduced root colonization by 72–91% and root discoloration (AUDPC values) by 57–70% (Table 3) in hydroponic chrysanthemums inoculated with *P. aphanidermatum* or *P. dissotocum*. Root discoloration, generally browning or graying in chrysanthemums, mark a transition from biotrophy to necrotrophy by *Pythium* and other Straminipiles (16,26,28), and was associated with deterioration in physiological functioning of the roots and reduced shoot growth in hydroponic pepper and lettuce (5,6,26). The strong correlations between bacterial suppression of root colonization by *P. aphanidermatum* or *P. dissotocum* and root discoloration in the chrysanthemums suggested that antagonism of pathogen growth and colonization in the roots is important for suppressing root discoloration and decay and thus for maintaining plant growth and productivity.

Among the bacterial strains evaluated, only *Ps. chlororaphis* 63-28 and *B. cereus* HY06 strongly suppressed both *P. aphanidermatum* and *P. dissotocum* in the chrysanthemum roots. Thus, in plants treated with the respective strains and inoculated with *P. aphanidermatum*, root colonization by the pathogen was 83% and 72% lower than in the pathogen controls, and AUDPC values were reduced by 61% and 65% (Table 3). For *P. dissotocum,* the respective strains reduced colonization by 70–90%. *Ps. chlororaphis* Tx-1 and *C. acidovorans* C-4-7-28 respectively strongly suppressed root colonization by *P. aphanidermatum* by 84% and 80% and AUDPC value by 66% and 57% (Table 3). However these strains did not significantly suppress colonization or root discolouration by *P. dissotocum* ($P<0.05$). In contrast, *B. gladioli* C-2-74 and *C. acidovorans* OCR-7-8-38, respectively, suppressed colonization by *P. dissotocum* by74% and 86%, and AUDPC values for the pathogen by 60% and 70%, but were ineffective against *P. aphanidermatum* (Table 3). Thus, suppressive activity of several of the bacterial strains was selective at the species level of the pathogen. Effectiveness of the other tested strains was generally low or not significant (Table 3). Strain effectiveness should be interpreted within the context of the conditions of the experiments, including the final density of strains introduced into the nutrient solution (usually 10^4 CFU ml^{-1}), timing

of treatments prior to pathogen inoculation, and the high inoculum density of the *Pythium* isolates employed (10^4 zoospores ml^{-1}). A perspective of effectiveness should also include consideration of the visual method used to estimate disease (root discoloration) and the destructive method used to estimate root colonization by the *Pythium* spp. at 18 days after inoculation. For example, pathogen incidence in root segments does not necessarily reflect density of pathogen hyphae in the roots. Nonetheless, the observations clearly demonstrated strong suppression of Pythium root rot under conditions that were favorable for rapid disease progress.

Effectiveness of the bacterial strains against *P. aphanidermatum* in the chrysanthemums was in several instances similar to findings in hydroponic pepper plants. *Ps. chlororaphis* TX-1, *Ps. chlororaphis* 63-28, *C. acidovorans* C-4-7-28, *B. gladioli* C-2-74 and *C. acidovorans* C-4-7-12 suppressed the pathogen in both hosts, but *Ps. fluorescens* HY05 was suppressive only in chrysanthemums. Other tested bacteria were only weakly suppressive or nonsuppresssive in each host. *Ps. chlororaphis* Tx-1 has an established record of effectiveness against *P. aphanidermatum* in hydroponic peppers, but consistently failed to reduce root discoloration associated with *P. dissotocum* (3,7). *Ps. chlororaphis* 63-28 has well-known activity against *P. aphanidermatum* in roots of cucumber and some other hosts (13).

Findings in the agar medium assay and in the biological control experiments with chrysanthemum plants suggested that antibiosis was a mode of action of some of the bacterial strains against the *Pythium* spp. and root discoloration. The suppression of pathogen growth on the agar medium can be interpreted in terms of inhibitory substances remaining in the medium after the cellophane membranes bearing the bacteria were removed. Lack of suppression, however, did not necessarily preclude antibiosis, for example by ephemeral or volatile substances. Changes in available nutrients in the medium during growth of the bacteria on the membranes were possibly of minor importance given the marginal suppression of *P. dissotocum* by several bacteria (*C. acidovorans* strains C-4-7-12, C-4-7-22, OCR-7-8-38 and OCR-7-8-39). *Ps. chlororaphis* 63-28 and *B. cereus* HY06, the only bacteria that markedly suppressed root discoloration caused by *P. aphanidermatum* and by *P. dissotocum*, each strongly suppressed radial growth of both of the pathogens on the agar medium. Antibiotic production was reported for *Ps. chlororaphis* 63-28 and strains of *B. cereus*, although not HY06 (13,20). Observations that *Ps. chlororaphis* Tx-1 strongly suppressed growth of *P. aphanidermatum* and moderately suppressed growth of *P. dissotocum* on the agar medium, roughly corresponded with the levels of suppression by this strain of root discoloration caused by the respective pathogens. These findings, and those of Chatterton *et al*. (3), may indicate that *P. dissotocum* has lower sensitivity than does *P. aphanidermatum* to phenazines or other antibiotics produced by *Ps. chlororaphis* Tx-1 (2,14). *B. gladioli* C-2-74 and *C. acidovorans* OCR-7-8-28 suppressed growth of both pathogens on the medium, and reduced discoloration in roots infected by *P. dissotocum*.

The observations on effects of treatment timing prior to inoculation with *P. aphanidermatum* (Table 6) clearly demonstrated the importance of early establishment of the bacteria in the root zone. While all four tested strains were effective in suppressing the pathogen when applied 14 days before inoculation, only the strains of *Ps. chlororaphis* were effective when applied at 7 days, and none was effective at 0 days. All of the stains may require a week or more in order to become sufficiently established on the roots to suppress attack by

high densities of *Pythium* zoospores. The strong correlations obtained between incidence of colonized root segments and discolored roots in the timing study and other experiments were consistent with the view that suppression of colonization is fundamental to controlling root browning, at least under conditions conducive to root discoloration (3,10,26).

Based on superior and generally consistent performance against *P. aphanidermatum* and *P. dissotocum* in chrysanthemums grown in the single-plant units, *Ps. chlororaphis* 63-28 and *B. cereus* HY06 have substantial potential for controlling Pythium root rot in commercial-scale hydroponic systems. These strains performed well even under conditions that were strongly favorable for the pathogens and root disease. For example, performance was strong despite the abrupt inoculation of roots with a high density of zoospores, and when the root zone temperature was high (32°C), which, from the present and earlier studies (26), is highly favorable for root necrosis caused by *P. aphanidermatum*. Effectiveness at high temperature would be important should Ontario growers adopt hydroponic methods for chrysanthemum production. Nutrient solution temperature should ideally be regulated near 20–22°C to minimize root browning, but this is often difficult to achieve in hydroponic systems currently used for various other crops in Ontario in which solution temperatures often reach 27–35°C, especially during sunny days in spring and summer. The data indicated that *Ps. chlororaphis* Tx-1 has major potential against Pythium root rot in commercial systems when the principal causal agent is *P. aphanidermatum*, but not when *P. dissotocum* is abundant. The strong activity of *B. gladioli* C-2-74 and *C. acidovorans* OCR-7-8-38 in several experiments (Table 3) suggests that these strains justify further study.

The data can be applied in developing protocols for employing the bacterial strains to protect chrysanthemums against Pythium root rot, including the cell density to use, and the mode and timing of applications. The ability of the bacterial strains to suppress *Pythium* and root rot when applied in the nutrient solution can be expected to facilitate any commercial use under hydroponic conditions, and suggests that cells of the bacteria in the nutrient solution are able to establish good associations with nearby chrysanthemum roots. Similar observations were made for *Ps. chlororaphis* Tx-1 and other bacterial strains in hydroponic peppers and cucumbers (3,7,30). Effects of rooting media used in hydroponic crops, such as coconut fiber and rockwool, on effectiveness of the bacterial strains against Pythium root rot in chrysanthemum, remain to be explored. The observations that the bacterial strains were generally more effective when applied well before (*e.g.* 14 days) roots were inoculated with *P. aphanidermatum*, indicated that protocols should require initial treatment early in root rot epidemics or before epidemics begin. Requirements for subsequent applications to hydroponic chrysanthemums have yet to be established, but findings in other hydroponic crops have indicated that only one to three applications of *Ps. chlororaphis* Tx-1, *Ps. chlororaphis* 63-28 and other strains are needed during crop cycles (*e.g.* 8,23,30). The present observations demonstrated that application of the superior bacterial strains to provide 10^4 CFU ml^{-1} nutrient solution, a level not difficult to achieve in commercial crops, is sufficient to strongly suppress Pythium root rot , although possible benefits of higher density justify investigation. Clearly, protocols developed based on data of the small-scale hydroponic units would need to be evaluated in representative commercial hydroponic systems that may be adopted for production of cut chrysanthemums, and continued throughout the crop cycle. Our observations (W. Liu and J.C. Sutton, 2005, unpublished) indicated that resistance of chrysanthemums to Pythium

root rot increases with age of the plants under hydroponic conditions. Given appropriate protocols, it may be possible to produce high quality flowering stems of chrysanthemum over long periods in hydroponic systems in Ontario greenhouses, and to take advantage of potential efficiencies of these systems compared with conventional ground beds.

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