

Evaluation of Rhizosphere Bacteria for Biological Control of *Pythium* Root Rot of Greenhouse Cucumbers in Hydroponic Culture

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ABSTRACT

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Five bacterial isolates were evaluated for their ability to reduce root rot caused by *Pythium aphanidermatum* (*Pa*) on cucumber (*Cucumis sativus* L. cv. Corona) grown under simulated commercial conditions in a rock wool hydroponic system. Two isolates of *Pseudomonas corrugata* (Pc13 and Pc35) and three of *Pseudomonas fluorescens* (Pf15, Pf16, and Pf27) were evaluated in both spring and early fall 1991 crops. Isolates Pc13, Pc35, and Pf15 were also evaluated in a 1992 spring crop. In all crops, 5-wk-old plants were set onto rock wool slabs and treated with water or 200 ml of a bacterial suspension (10^6 cells per milliliter). One-half of the plants were inoculated with 10^5 zoospores of *Pa* strain 186. In 1991, *Pa* inoculum was applied 6 days after treatment with biocontrol agents. In 1992, it was applied three times: 1 wk before, at the same time, and 1 wk after treatment with the biocontrol agents. In the 1991 spring crop, inoculated plants treated with Pc13 or Pf15 produced 88% more marketable fruit than the inoculated control ($0.10 > P > 0.05$, orthogonal contrasts). In the absence of the pathogen, these two isolates significantly increased the number of fruit (32 and 41%, respectively), compared to the noninoculated control ($P < 0.05$). In the fall crop, treatment of *Pa*-inoculated plants with Pc13 or Pf15 significantly increased marketable fruit production (almost 600% over that of the inoculated control). Pc13 and Pf15 also increased total fruit weight produced by inoculated plants. These isolates significantly increased the shoot dry weight in plants not inoculated with the pathogen. In both crops, treatment with any of the bacterial isolates resulted in significantly reduced cull rates compared to the *Pa*-inoculated control. Disease development in the 1992 crop was lower than in the previous year, and the effect of treatments with biocontrol agents was not significant. However, treatment with Pf15 alone resulted in significantly greater fruit production compared to plants not inoculated with *Pa* or treated with biocontrol agents.

Pythium spp. are the root pathogens most commonly encountered, and often the most serious, in hydroponic production of seedless hybrid long cucumber (*Cucumis sativus* L.) (1,5,12,23). Of the numerous *Pythium* species that can cause root rot of greenhouse cucumber, *P. aphanidermatum* (Edson) Fitzp. is the most widely reported. *Pythium* spp. can be easily introduced to these systems from infested water sources (16), contaminated soil (22,24), or naturally infested peat-based propagation media (5). Fungus gnats (*Bradysia impatiens*) (7) and shore flies (*Scatella stagnalis*) (9) may also be involved in the introduction and spread of *Pythium* in commercial greenhouses.

Pythium spp. have a poor competitive ability in the soil relative to other root-colonizing organisms and often act only as primary colonizers (13). However, in hydroponic production systems, low populations of competing microbes and the efficient dissemination of zoospores through the nutrient solution increases the potential for disease development (3,12,18,28,33). Following infection,

Pythium spp. may exist in the cucumber root system as minor pathogens until the plant is stressed during fruiting (5). Pathogenesis can be rapid, with extensive root and stem rotting occurring within 3-9 days after introduction of the pathogen (8,11,12). Severely affected plants are poorly anchored, and daytime wilting often occurs.

Currently, no fungicides are registered for use against *Pythium* spp. on greenhouse cucumber in Canada, although acylalanine fungicides have shown promise (19). New registrations allowing addition of fungicides to the nutrient solution may be difficult due to phytotoxicity (27) and the potential for unacceptable residues in the fruit (28). A metalaxyl drench ($10 \mu\text{g a.i./ml}$) controlled plant mortality from *P. aphanidermatum*, but it was only fungistatic and the pathogen could be recovered from treated plants (5). Addition of surfactants to the nutrient solution at 20-25 $\mu\text{g/ml}$ caused zoospore lysis and inhibited zoospore formation in *Pythium* spp. (26). UV irradiation (16,25), filtration of the nutrient solution (11), and manipulation of light, temperature, or nutrient composition (6,8) have also reduced disease severity. Amendment of the nutrient solution with potassium silicate reduced root decay and yield loss in long English cucumber infected with

Pythium ultimum (2). Resistance is not available in commercial hybrid seedless cucumber cultivars, nor is it likely to be available in the foreseeable future due to the small potential market for this crop (32).

The introduction of biocontrol agents to hydroponic or soilless substrates to control *Pythium* diseases has received little previous attention, although some research has targeted *Fusarium* diseases (4,14). This paper presents the results from evaluation of five bacterial antagonists tested on seedless hybrid cucumbers in a greenhouse production system for control of *P. aphanidermatum*.

MATERIALS AND METHODS

Production of bacterial and fungal inoculum. Two isolates of *Pseudomonas corrugata* (Pc13 and Pc35) and three isolates of *P. fluorescens* (Pf15, Pf16, and Pf27) were previously selected from in vitro and in vivo screening of 600 rhizobacterial isolates from cucumber roots (17). They were stored in nutrient broth with 10% glycerol at -80°C . Bacterial cultures were prepared in seeding flasks containing 250 ml of nutrient broth with a loop of bacteria from the frozen cultures. The cultures were incubated on a rotary shaker (100 rpm) at 24°C for 24 hr. Bacterial cells were washed by centrifugation at 2,000 g for 10 min, and the pellet was resuspended in 0.1 M MgSO_4 . Density of the bacterial suspension was adjusted to 10^6 cells per milliliter by measuring absorbance at 640 nm and comparing this data to a standard curve.

P. aphanidermatum (*Pa*) isolate 186 (W. Jarvis, Agriculture Canada, Harrow, Ontario) was used in all experiments. The fungus was grown on V8 agar, and zoospores were produced according to the method of Paulitz et al (17). Zoospore density was adjusted to 500 zoospores per milliliter for inoculation of rock wool slabs.

Production of seedlings. Cucumber cultivar Corona was seeded into plug trays containing medium-grade granular rock wool. Seedlings were grown for 7 days on a growth bench (26°C) with 16 hr of light (combined incandescent-fluorescent, $150 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) per day. The plants were then set into $10 \times 10 \times 6.5$ cm rock wool cubes previously saturated with a nutrient solution containing Peter's 10-52-10 soluble fertilizer (2 g/L). The plants were grown in the cubes for

4 wk and were watered daily to saturation with alternating tap water and the nutrient solution.

Greenhouse conditions. A 30 × 5.5 m quonset house covered with clear plastic was used for all experiments. In the spring and fall 1991 crops, random fiber rock wool slabs measuring 100 × 7.5 × 20 cm were placed in a double-row configuration with the slabs butted together end-to-end in the row. Vertical fiber slabs of the same dimensions were used for the spring 1992 crop. All slabs were presoaked with nutrient solution (1.26 g/L Peter's Hydrosol + 0.83 g/L CaNO₃) for 3 days to adjust the pH to 6.5 prior to setting out plants. Two plants in rock wool cubes were set on each slab to provided a final spacing of 0.6 m² per plant.

During the cropping period the slabs were irrigated and fertilized with nutrient solution (Peter's Hydrosol and CaNO₃). Stock solutions were diluted 1:100 at the point of delivery to the slabs with Dosmatic Plus liquid dispensers (J.F. Equipment Co., Dosmatic International, Lewisville, TX 75067). An output pH of 6.5 and a slab electrical conductivity of 2.3 mS were maintained during the cropping period. Nighttime supplemental heating was provided as required. Treatments were applied in a randomized complete-block design. A total of 200 ml of bacterial suspension (10⁶ cfu/ml) was added to each cube 2 and 4 days after placing the plants on the slabs. Six days after the second application of biocontrol agents, 200 ml of a zoospore suspension of *Pa* (500 zoospores per milliliter) was flooded onto the surface of each cube.

Table 1. Effect of bacterial isolates on percentage of unmarketable or cull fruit of cucumber cultivar Corona inoculated or not inoculated with *Pythium aphanidermatum*, crops 1 and 2, spring and fall 1991

Treatment	Percent cull	
	Crop 1	Crop 2
None	8 b ^y	0 b
<i>Pythium</i>	44 a	25 a
13	10 b	0 b
13 + P ^z	6 b	0 b
15	7 b	0 b
15 + P	8 b	0 b
16	5 b	0 b
16 + P	10 b	0 b
27	5 b	0 b
27 + P	13 b	0 b
35	4 b	0 b
35 + P	7 b	0 b

^yTreatments followed by the same letter are not significantly different at $P \leq 0.05$. Cull fruit were less than 28 cm in length or had a bend, crook, or bulge greater than the diameter of the fruit.

^z+ P = inoculated with *P. aphanidermatum*. Average values are from three rock wool slabs in the first crop and four slabs in the second crop, two plants per slab. Harvest period was 4 wk in the first crop and 1 wk in the second.

The fertigation pumps were shut off for 12 hr following the introductions of *Pa* and bacteria.

Fruit was harvested daily during the cropping period and was considered mature when it had attained a diameter of 4.5 cm at any point along its length. They were individually weighed and graded according to commercial standards. Fruits that were soft at harvest or had severe deformities, including multiple bulges or underdeveloped blossom ends, were considered unmarketable (cull grade). Fruit damaged by abrasion or curving as a result of contact with the crop support wires were not culled. Plant wilting and mortality were recorded when visible. The degree of wilting was determined by daily examination of the plants in the late afternoon, and was rated as follows: 0 = no wilting; 1 = very slight wilting, leaf blade with loss of turgor but no significant drooping of margins; 2 = leaves drooped slightly at margin, but petiole unaffected and plant recovered overnight; 3 = the entire blade drooped and petiole with loss of turgor, plant recovered the next day if conditions were overcast or cool; 4 = both leaf blades and petioles collapsed, plants did not recover; and 5 = dead plant. Leaf production and leaf diameter were measured, and plants were examined periodically for fruit abortion. At the end of the experiment, plant shoots were dried for 4 days at 70 C, and dry weights were determined. Plant roots were examined for signs of necrosis.

Crop 1, spring 1991. Twelve treatments, each with three replications of two plants per slab, were included: biocontrol agent Pc13, Pf15, Pf16, Pf27, or Pc35 alone; biocontrol agents with *Pa*; *Pa* without biocontrol agents; and a noninoculated control (no added *Pa*, no biocontrol agent).

Crop 2, fall 1991. The biocontrol agent Pc13, Pf15, Pf16, Pf27, or Pc35 alone, and each in combination with *Pa* were used, each with four replications of two plants. There was also a *Pa*-inoculated treatment without biocontrol agents and a noninoculated control without *Pa* or biocontrol agents.

Crop 3, spring 1992. The three most effective bacterial isolates (Pc13, Pf15, and Pc35) from 1991 were tested, alone and in combination with *Pa*. The pathogen was inoculated on three different dates, 1 wk before, the same day, and 1 wk after treatment with the biocontrol agents. A *Pa*-inoculated treatment without biocontrol agents was included for each inoculation time. One treatment without *Pa* or biocontrol agents was included for the entire experiment.

Monitoring bacterial population densities. A small sample of rock wool and root material (approximately 1 g) was removed with a sterile forceps from each slab 3 and 7 wk after the bacteria were introduced. These samples were

placed in 125 × 16 mm test tubes containing 9 ml of sterile distilled water and were sonicated for 30 sec and vortexed. Since all bacterial isolates used in the greenhouse experiments were spontaneous mutants selected for resistance to rifampicin, the solutions were serially diluted and plated on nutrient agar amended with 100 µg/L rifampicin. Plates were incubated at 26 C for 48 hr, and the number of colonies per plate was counted and expressed as cfu/g dry weight of rock wool plus root material.

Monitoring *Pythium* population densities. Samples of rock wool containing roots were removed from each treatment and placed in 125 × 16 mm test tubes containing 9 ml of sterile distilled water and 20 pearl millet seeds. The sample was placed in the tube so that it was in contact with the water surface but not fully immersed. This allowed zoospores to swim to the millet seeds but prevented direct contact between the sample and the seeds. The test tubes were incubated for 24 hr at 26 C. Then the seeds were plated on *Pythium*-selective medium (15). The number of infected seeds was determined after 48 hr and compared to a standard curve relating the incidence of colonization to zoospore population density (20).

Statistical analyses. The results of all greenhouse experiments were analyzed by analysis of variance, with *Pythium* and bacterial treatments as separate variables. Treatment means were separated using Duncan's multiple range test. Orthogonal contrasts with *F* tests were also used to make the following comparisons: *Pa*-inoculated control vs. *Pa*-inoculated plants treated with isolates Pc13 and Pf15 and the noninoculated control vs. noninoculated plants treated with Pc13 and Pf15. In crop 3, *Pythium* inoculation time was also included as a variable. The wilt severity data were analyzed by the Kruskal-Wallis one-way analysis of variance, a nonparametric rank test. Plant mortality data were analyzed with Cochran's *Q* test.

RESULTS

Crop 1, spring 1991. Late-afternoon wilting occurred almost 2 wk earlier in *Pa*-inoculated treatments than in treatments not inoculated with *Pa*. The most severe wilting occurred in the *Pa*-inoculated treatment without biocontrol agents, while treatments with isolate Pc13, Pf15, or Pf27 and *Pa* inoculation showed significantly less wilting at the end of the experiment (Table 1). Isolate Pc13 also reduced wilt 12 days prior to the termination of the experiment. When treatments were sampled 2 wk after *Pa* inoculation, more than 300 zoospores per milliliter were recovered from all *Pa*-inoculated treatments, while *Pa* was not recovered from either the treatment without *Pa* or biocontrol agents or the treatments that received the biocontrol

agents alone. Root discoloration occurred in all *Pa*-inoculated plants, but they remained well anchored to the slabs. No root discoloration was seen in the noninoculated plants. Three weeks after application of the biocontrol agents, population densities in the slabs ranged from 5×10^5 to 1×10^7 cfu/g dry weight of sample. Biocontrol agents were not recovered from the treatment not inoculated with *Pa* or biocontrol agents, or from the *Pa*-inoculated treatment without biocontrol agents.

Inoculation with *Pa* significantly reduced the total number of marketable fruit produced per rock wool slab over the harvest period (Fig. 1A). Treatment of noninoculated plants with isolates Pc13 and Pf15 significantly increased fruit production by 32 and 41%, respectively ($P < 0.05$, orthogonal contrasts). *Pa*-inoculated plants treated with isolates Pc13 and Pf15 also increased fruit production by 88%, but the increase was not significant at the 0.05 level ($0.10 > P > 0.05$, orthogonal contrasts). Inoculation with *Pa* significantly reduced shoot dry weight, but treatment with biocontrol agents had no effect (Fig. 1B). Treatment with all biocontrol agents significantly reduced the percentage of non-marketable or cull fruit (Table 1). *Pa* inoculation also reduced the total weight of fruit produced from 13.3 to 5.3 kg per slab, but treatment with biocontrol

agents had no effect (*data not shown*). There were no significant interactions between *Pa* inoculation and treatment with biocontrol agents for any of the parameters measured.

Crop 2, fall 1991. Late afternoon temperatures consistently exceeded 30 C and often reached 35–37 C in this experiment. Slab temperatures ranged from 25 to 30 C. Plant growth under these conditions was rapid, and the increased water demand caused more rapid wilting in all plants and higher plant mortality than had occurred in the first crop. There was no significant difference in mortality ($P \geq 0.05$, by the Cochran *Q* test) between the *Pa*-inoculated treatment without biocontrol agents and the treatments receiving a biocontrol agent and *Pa*. Only slight wilting occurred in treatments not inoculated with *Pa*, and the wilt indices rarely exceeded 1. On 2 September, 1 wk before the end of the experiment, the plants inoculated with *Pa* but not treated with biocontrol agents showed greater wilt (3.75) and the plants treated with Pc13 showed the least wilt (1.75) among the inoculated treatments, but these differences were not significant (Table 2). At the end of the experiment, the wilt severity indices in all *Pa*-inoculated treatments exceeded 2.5, with no significant differences among the treatments. Treatment with some biocontrol agents delayed severe wilting and plant death. On 1 September, 5/8 of the plants in the *Pa*-inoculated treatment with biocontrol agents had died. In treatments inoculated with *Pa* and treated with Pc13, Pf15, or Pf27, the mortality was 3/8, 1/8, and 3/8 of the plants, respectively. By 9 September, mortality rates had increased to 7/8 of the plants in the *Pa*-inoculated treatment without biocontrol agents, compared to 4/8, 5/8, and 4/8 of the *Pa*-inoculated plants treated with isolate Pc13, Pf15, or Pf27, respectively. Plants from all treatments inoculated with *Pa* had discolored root systems, and many of the surviving plants were poorly anchored to the slabs. The roots of plants

from the *Pa*-free treatment without biocontrol agents or from the treatments with the biocontrol agents alone were unaffected.

Inoculation with *Pa* significantly reduced fruit production (Fig. 2A). When *Pa*-inoculated plants were treated with isolates Pc13 or Pf15, production of marketable fruit was significantly increased (five- to sixfold, $P < 0.01$, orthogonal contrasts). These isolates also increased the total fruit weight produced by *Pa*-inoculated plants (317 and 280%, respectively, $P < 0.05$, orthogonal contrasts, Fig. 2B). The biocontrol agents did not increase fruit production of noninoculated plants. The shoot dry weights of *Pa*-inoculated plants were not significantly affected by any of the treatments with biocontrol agents (Fig. 2C). However, the shoot dry weight of noninoculated plants was significantly increased by treatment with Pc13 and Pf15. Treatment of *Pa*-inoculated plants with all biocontrol agents significantly reduced the percentage of nonmarketable fruit from 25 to 0% (Table 1). No statistical interaction was detected between *Pythium* and biocontrol agent treatments.

At the end of the experiment, 100–300 zoospores per milliliter were present in all replications of all *Pa*-inoculated treatments. *Pa* was also recovered at a low level from one replication of the *Pa*-free treatment without biocontrol agents, but was not recovered from any treatments that received biocontrol agents alone. On 18 September, population densities of the biocontrol agents ranged from 10^5 to 10^7 cfu/g dry weight of sample. Biocontrol agents were also recovered from one replication of the *Pa*-inoculated treatment without added biocontrol agents and from three replications of the control without added *Pa* or biocontrol agents. High temperatures combined with algal growth in gutters and under slabs resulted in a high population of fungus gnats during this crop, which may have contributed to the spread of biocontrol

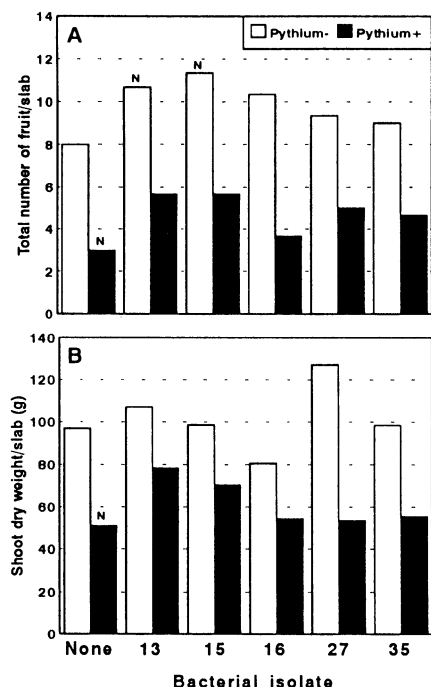


Fig. 1. Effect of bacterial isolates on (A) total fruit production and (B) shoot dry weight of cucumber cultivar Corona inoculated or not inoculated with *Pythium aphanidermatum*, crop 1, spring 1991. Values represent marketable fruit and dry weight produced per rock wool slab over the entire harvest period, two plants per slab, three slabs per treatment. Bars marked "N" are significantly different from the noninoculated control (None, *Pythium*-), according to orthogonal contrasts, $P < 0.05$.

Table 2. Effect of bacterial isolates on the wilt severity indices of cucumber cultivar Corona inoculated with *Pythium aphanidermatum*

Treatment	Average wilt indices ¹			
	Crop 1		Crop 2	
	June 5	June 17	Sept. 2	Sept. 9
None	0* ²	0*	0.25*	0.25*
<i>Pythium</i> only	2.50	2.33	3.75	3.50
Pc35 + P	1.83	1.16	2.50	2.75
Pf15 + P	0.66	0*	2.75	3.62
Pf16 + P	1.50	1.00	2.50	2.87
Pf27 + P	1.00	0*	2.25	2.87
Pc13 + P	0*	0*	1.75	3.50

¹Wilt severity indices were determined as follows: 0 = no wilting; 1 = very slight wilting, leaf blade loss of turgor, no significant drooping of margins; 2 = leaves drooping slightly at margin, petiole unaffected, plant recovered overnight; 3 = blade drooped, petiole with loss of turgor, plant recovered if next day was overcast or cool; 4 = leaf blade and petioles collapsed, plants did not recover; and 5 = plant dead.

²Treatments within each column marked * are significantly different from the *Pythium* only treatment, according to Kruskal-Wallis one-way analysis of variance, $P \leq 0.05$.

agents and *Pa* to the control treatments. Ventilation system failure on 9 and 10 September resulted in high-temperature (45 C) injury to the plants, so the trial was terminated after only 4 wk in the greenhouse.

Crop 3, spring 1992. The data for all plant growth and harvest parameters were analyzed using biocontrol agent and *Pa* inoculation date as main effects. Neither of these factors was significant, nor was there any significant ($P \leq 0.05$) interaction between the two. Shoot dry weight among treatments was not significantly different in this crop. Disease development was slower than in the spring 1991 crop, although slab temperatures were similar (20–25 C). Plant

wilting in all treatments was very slight. *Pa* recovery from all inoculated slabs was low, with zoospore population densities below the detection threshold of the millet baiting method. However, *Pa* was recovered from all treated slabs by direct plating of root samples on *Pythium* selective medium. The population density of biocontrol agents in the slabs at the end of the harvest period ranged from 5×10^3 cfu/g to levels that were below the detection threshold at the dilutions used.

Fruit number and percent nonmarketable fruit were not significantly decreased by inoculation with *Pa* in this crop. However, treatment with isolate Pf15 alone (no *Pa*) resulted in significantly greater fruit numbers and fruit weight than did the *Pa*-free treatment without biocontrol agents. The *Pa*-free treatment without biocontrol agents yielded 12.4 kg and 26.2 fruit per slab, while the slabs treated with Pf15 yielded 15.9 kg and 33 fruit per slab.

DISCUSSION

The strategy of treating plants produced in hydroponic systems with biocontrol agents as a prophylactic treatment offers several advantages (21). These substrates have a lower microbial content than soil, reducing the competition for establishment of biocontrol agents and allowing biocontrol agents with a low competitive ability to become established. These substrates are used in controlled environments, eliminating much of the variability that reduces the effectiveness of biocontrol agents in the field. The high economic value of greenhouse crops and the ease of introducing the biocontrol agent into the system are added benefits.

Little previous research has been reported on successful biocontrol of *Pythium* in soilless substrates. This is the first report of reduction of a *Pythium* disease with rhizobacteria in rock wool. Our research showed that isolates of *Pseudomonas* selected by in vitro and in vivo screening techniques (17) had an effect on *P. aphanidermatum* under near commercial conditions in two out of three crops, the two with highest disease pressure. Some previous attempts to control *Pythium* on lettuce and cucumbers in a hydroponic system were not as successful (10). However, *Pseudomonas* spp. did reduce Fusarium wilt of carnations grown in rock wool (29).

In our work, differences were seen in the rate and severity of wilting among treatments. In both greenhouse crops 1 and 2, the highest level of wilting was seen in the *Pa*-inoculated treatment without biocontrol agents. Lower levels of wilting occurred in treatments where biocontrol agent Pc13, Pf15, or Pf27 was applied prior to inoculation with *Pa*. Infection by *Pa* had a more obvious effect on plant yield in terms of number of marketable fruit, percentage of culls, and total weight of fruit produced. Treatment

with biocontrol agents Pc13 and Pf15 prior to inoculation with *Pa* increased the number of marketable fruit, an increase that was statistically significant in the second crop and almost significant in the first crop (Figs. 1A and 2A). In both cases, fruit numbers were increased to a level intermediate between those of the plants in the *Pa*-inoculated treatment without biocontrol agents and plants in the *Pa*-free treatment without biocontrol agents. When the fruit was graded, the cull rate in the *Pa*-inoculated treatment without biocontrol agents was significantly higher ($P \leq 0.05$) than in the *Pa*-free treatment without biocontrol agents, but treatments where the biocontrol agents had been applied prior to inoculation with *Pa* were not significantly different from the *Pa*-free treatment without biocontrol agents (Table 1). Cucumbers tend to respond to stress initially by compromising fruit production. When the stress is not sufficient to cause abortion, both fruit development rate (indicated by fruit number) and fruit quality are compromised.

Disease development was greater in the fall crop than in either spring crop. Higher slab temperatures that were closer to the optimum for growth of *Pa* increased disease pressure in the fall, and daytime air temperatures were stressful to the growth of cucumbers. Overall disease was lower in the spring 1992 crop than in the spring 1991 crop. This could be attributed to slightly earlier *Pa* inoculation times, which corresponded to lower slab temperature or to the use of rock wool slabs with a vertical fiber orientation. Random fiber blocks were used in the 1991 crops. The vertical fiber orientation may improve drainage, making conditions less favorable for *Pa*. The recovery of *Pa* from the slabs in this crop was lower than in either 1991 crop. Further studies into the effect of slab orientation on *Pythium* growth could be useful.

Although the precise mode of action of the potential biocontrol bacteria used here is beyond the scope of this work, plant growth promotion may be at least partially responsible for the activity of Pc13 and Pf15. These isolates increased fruit production in noninoculated plants in the spring 1991 crop and increased shoot dry weight in the fall crop. In previous test tube studies and initial screening, Pc13 and Pf15 significantly increased plant growth over that of an untreated control (17,20). In the greenhouse, treatments with Pf15 in the absence of *Pa* resulted in higher yields, while treatments with isolate Pf15 reduced the effects of *Pa* inoculation. Van Peer et al (30) also reported an increased growth response of cucumber from *Pseudomonas* spp. in hydroponic culture. Isolate Pf15 may reduce disease losses by increasing plant vigor and reducing the susceptibility of the plant

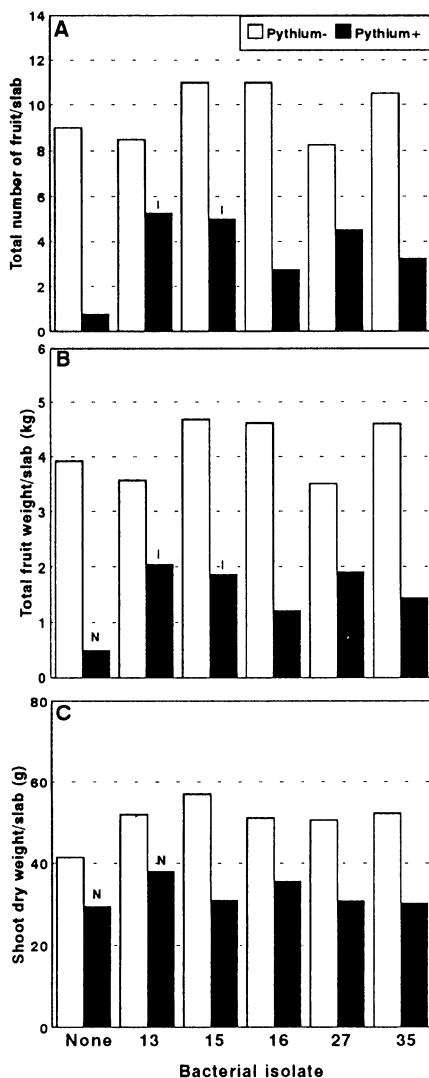


Fig. 2. Effect of bacterial isolates on (A) fruit production, (B) total fruit weight, and (C) shoot dry weight of cucumber cultivar Corona inoculated or not inoculated with *Pythium aphanidermatum*, crop 2, fall 1991. Values represent averages per rock wool slab over the entire harvest period, two plants per slab, four slabs per treatment. Bars marked "N" and "I" are significantly different from the noninoculated (None, *Pythium*-) and inoculated (None, *Pythium*+) controls, respectively, according to orthogonal contrasts, $P < 0.05$.

to stress and subsequent damage from the *Pa. Pythium* is often most damaging to plants under stress conditions (3). These bacterial isolates may also induce systemic resistance to *P. aphanidermatum* in the root system (35). Studies with these bacterial isolates on split root systems showed less root loss when the opposite root system was treated with isolates Pc13 and Pf15, even though there was no physical contact between the bacteria and pathogen (35). Wei et al (31) also reported systemic resistance to a foliar pathogen in cucumber induced by plant growth-promoting rhizobacteria applied to the root. Finally, competition for nutrients in root exudates and interference with zoospore chemotaxis and encystment may also be a mechanism of biocontrol by these isolates (34). Additional research is needed to test these isolates on other cucumber cultivars, other *Pythium* spp., and other growing media. Improvements in the method of application are also needed, for example, through the irrigation system.

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