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Evaluation of plant growth-promoting rhizobacteria for biological control of Pythium root rot of cucumbers grown in rockwool and effects on yield

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Abstract

Three strains of *Pseudomonas fluorescens* (63-49, 63-28, and 15), one strain of *Pseudomonas corrugata* (13) and one strain of *Serratia plymuthica* (R1GC4) were tested on rockwool-grown cucumbers for their ability to reduce Pythium root-rot caused by *Pythium aphanidermatum*. These strains were previously selected for biocontrol ability from collections of >4000 bacteria. Strains 63-49 and 63-28 were tested on cucumber plants grown in rockwool in two replicated *Pythium*-inoculated trials conducted in British Columbia (B.C). Another inoculated, replicated trial was conducted in Quebec with all five strains. Cucumber yields (fruit number and weight) were measured over a ten-week harvest period. Strain 63-49 caused an early promotion of plant growth and increased cucumber yields at early harvests. No measurable effect of *Pythium* inoculation on disease development was observed in the Quebec trial, due to unfavourable cool weather. However, 63-49 significantly increased the total number of cucumbers (12%) and cucumber weight (18%), compared to the non-treated control. Strains 13, 15 and R1GC4 slightly increased the cumulative cucumber yields, but strain 63-28 had no effect. In the B.C. trial, inoculation with *P. aphanidermatum* reduced the number and weight of cucumbers by 27%. Treatments of *Pythium*-inoculated cucumbers with 63-49 significantly increased fruit number and weight by 18%, compared to the *Pythium*-inoculated control. Strain 63-28 increased the cumulative number of cucumbers over time, compared to the *Pythium*-inoculated control, but the increase was less than with 63-49. The use of *Pseudomonas* spp. in rockwool-grown cucumbers can increase yields, both in the presence and absence of Pythium root rot, and with variable seasonal conditions and disease pressures.

Introduction

Long English cucumber (*Cucumis sativus* L.) is an important crop in the greenhouse vegetable industry in Canada, with revenues of $43.3 million in 1992 (Anonymous, 1992). Most of the production occurs in the provinces of Ontario and British Columbia (B.C.), and to a lesser extent in Quebec, Alberta and the Maritimes.

Cucumber plants are grown in soilless media, eg: rockwool, peat or nutrient film, to provide optimal conditions for growth and yield. In British Columbia, 10-L plastic bags containing Douglas fir (*Pseudotsuga menziesii*) and Western hemlock (*Tsuga heterophylla*) sawdust are commonly used (Mass and Adamson, 1981; Anonymous, 1993). These media provide a conducive environment for dissemination and establishment of pathogens such as *Pythium* species. Crown rot of greenhouse cucumbers caused by *Pythium* species is characterized by a rot of the basal stem and crown of the plant, accompanied by wilting and reduced growth and fruit production (Menzies and Jarvis, 1994).

With severe infection, the plants may die. Symptoms generally develop at early fruit set (4–8 weeks after transplant) and are accentuated during hot weather and water stress. The pathogen also rots roots, resulting in stunting and yield reduction.

The causal agents of *Pythium* root and crown rot on cucumber in British Columbia are *Pythium aphanidermatum*, *P. irregulare* and *Pythium* sp. Group G., with *P. aphanidermatum* being the most important (Favrin et al., 1988). *Pythium ultimum* and *P. aphanidermatum* are the dominant pathogens in Quebec greenhouses (Paulitz et al., 1992). In a pathogenicity study of *Pythium* spp. on cucumber in rockwool in France, only *P. aphanidermatum* caused plant death, root decay, and reduction of yield (Moulin et al., 1994b)

Control of *Pythium* spp. in greenhouses involves the use of fungicide drenches, eg: captan and thiram at planting time, in addition to treatment of seeds. If symptoms are observed later in the season, growers will often replant. Fungicides are not registered for use on cucumbers grown in soilless culture, and may cause phytotoxicity under these conditions. Since losses due to *Pythium* crown rot can approach 25–30%, there is considerable incentive to develop alternative disease control strategies. Previous research has demonstrated the ability of several biological control agents to protect against seed rots and damping-off caused by various *Pythium* spp. (Elad and Chet, 1987; Hadar et al., 1983; Paulitz and Baker, 1987). However, the potential for using bacterial species as biocontrol agents on mature cucumbers grown in soilless culture has not been extensively explored. Rankin and Paulitz (1994) showed that two species of *Pseudomonas* could increase fruit production and weight of *Pythium*-inoculated cucumber plants grown in rockwool. The objective of the present study was to evaluate several strains of bacteria for disease-reducing capability on cucumbers grown under commercial conditions. Replicated trials were conducted at three locations in Canada over a period of 3 years. Detailed yield data was recorded over the entire harvest period to quantify the effect of *Pseudomonas* spp. on the dynamics of fruit production, both in the absence and presence of *Pythium*, and under variable seasonal conditions and disease pressures.

Materials and methods

Fungal and bacterial inoculum

Pythium aphanidermatum, isolated from diseased greenhouse cucumbers in 1991 by J. Menzies, was grown in V8-cholesterol broth, rinsed twice with distilled water (DW) and blended for 10 sec in 20 ml DW. This inoculum was used in the B.C. trials. For the Quebec trial, *Pythium aphanidermatum* isolate 186 (W. Jarvis, Agriculture Canada, Harrow, Ontario NOR 1GO) was grown on V8 agar and zoospores were produced according to the method of Paulitz et al. (1992).

Pseudomonas fluorescens strains 63-28 and 63-49 isolated from canola roots in Manitoba (Kloepper et al., 1988) were grown in nutrient broth for 24 h at 28 °C on a rotary shaker at 150 rpm. One ml of each culture was pipetted onto two plates of Pseudomonas Agar F. After 48 h, bacterial suspensions were made by pouring a few ml of sterile distilled water (SDW) onto the plates, loosening the bacteria with a sterile rubber scraper and adding the suspension to 150 ml SDW. *P. fluorescens* strain 15 and *P. corrugata* strain 13 isolated from soil in Quebec (Paulitz et al., 1992), *Serratia plymuthica* strain R1GC4 (G. Brown, Agrium Inc., Saskatoon, Saskatchewan S7N 2X8) strains 63-28 and 63-49 were grown in tryptic soy broth for 48 h. Inoculum was prepared by diluting suspensions 1:10 with 0.1 M $MgSO_4$. The final inoculum contained approximately 10^8 cfu/ml.

Biocontrol of crown rot in soilless culture. Summerland and Agassiz, British Columbia. Seeds of cucumber (*Cucumis sativus* L.) cv. Fidelio (Deruiter Seeds, Columbus, Ohio, USA) were soaked in 50 ml of a suspension of *P. fluorescens* strains 63-28 and 63-49 (10^8 cfu/ml) or in SDW for 7 min. The seeds were dried on filter paper in petri plates in a laminar flow hood for 3 h. Ten ml of SDW were added to the seeds and the petri plates were placed in the greenhouse at 28 °C with a 16-h photoperiod. After the seeds had germinated, the seedlings were transferred to 10×6.5×10-cm rockwool cubes on plastic saucers. Each seedling was placed in vermiculite in a 2-cm well cut into the rockwool cube, which was watered from below. After the cotyledons had fully expanded, the greenhouse temperature was lowered to 24 °C and halogen lighting was provided 24 h per day. On day 9, 40 ml of a bacterial suspension (10^8 cfu/ml) was added to half of the cubes, to give a concentration of 6.1×10^6 cfu/cm^3 rockwool. On day 18, the plants were inoculated with

10 ml of *P. aphanidermatum* inoculum. Control plants were drenched with an equal volume of DW. When the plants reached the four-leaf stage (35 days after seeding), they were transplanted into individual 5-L plastic bags containing sawdust and inoculated once more with bacteria and *P. aphanidermatum*. Each plant received the complete nutrient solution as described in the production guide for commercial growers (Anonymous, 1988). Fertilizer applications were made eight times a day, for 10 min per application. Each plant received 0.87 L at each application or 7 L per day. The plants were supported using nylon cord that was attached to a horizontal wire 2 m from the ground. The plants were trained using the "umbrella" system (Klieber et al., 1993) and fruit were allowed to develop on every node starting at the 9th node from the base of the plant. Plant height was measured when plants were 29, 42, and 54 days old. Cucumbers were harvested and weighed when they had reached a diameter of 4.5 cm. In the Summerland, B.C. trial, there were nine replicate plants for each of the four treatments (untreated, strain 63-28 with *P. aphanidermatum*, strain 63-49 with *P. aphanidermatum*, and *P. aphanidermatum* alone). Fresh and dry weights of shoots of all plants were measured nine days after harvest had begun. The same treatments were used in the Agassiz trial, with 14 replicate plants per treatment. The Agassiz, B.C. trial began 22 Dec 1993 and ended 25 May 1994. Measurements made included number of fruit per plant per harvest, fruit grade, and fruit fresh weight. Both experiments in British Columbia were arranged in the greenhouse in a completely randomized design.

Quebec. Cucumber (cv. Corona) seeds were planted in loose rockwool in plug trays and germinated on a growth bench at 27 °C with a 16-h photoperiod. After the cotyledons had expanded, seedlings were transplanted into 10×6.5×10-cm rockwool cubes on plastic trays and watered daily with Peter's 10-52-10 (2g/L). On day 10, 100 ml of bacterial suspension (10^8 cfu/ml) of strains 63-28, 63-49, R1GC4, 13 and 15 were added to each plant. An equal volume of water was added to control plants. After one month, seedlings were transplanted onto rockwool slabs (100×7.5×20-cm) which had been soaked for 48 h in a complete nutrient solution (1.26 g/L Peter's Hydrosol + 0.83 g/L $CaNO_3$). Slabs had been arranged in four rows in a 30×5.5 m quonset house covered with plastic. Treatments were applied in a randomized complete block design. There were 12 treatments with 6 replicates, each replicate consisting of one slab with two plants.

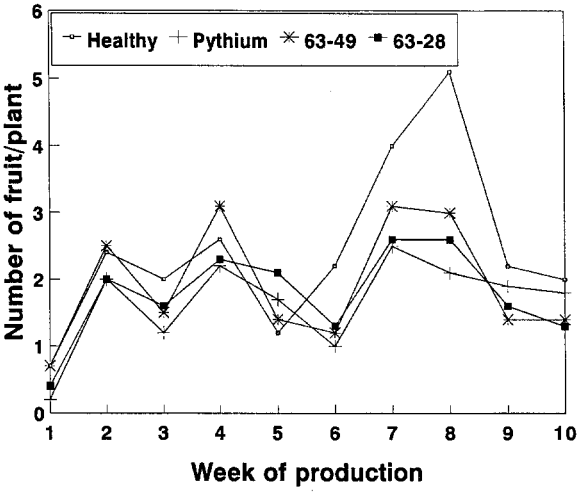

Figure 1. Effect of bacterial treatments and inoculation with *Pythium aphanidermatum* on weekly yield (number of cucumbers) of plants grown in rockwool, Agassiz trial. Healthy: no bacterial treatment or *Pythium* inoculation; *Pythium*: no bacterial treatment, inoculated with *Pythium aphanidermatum*; 63-49: treated with strain 63-49 prior to *Pythium* inoculation; 63-28: treated with strain 63-28 prior to *Pythium* inoculation.

Stock solutions of fertilizer were diluted 1:100 with Dosmatic Plus pumps (J.F. Equipment Co., Dosmatic International, Lewisville, TX 75067, USA). Plants were supported and pruned as described above. The following twelve treatments were used: each of the five bacterial isolates alone, each bacterium with *P. aphanidermatum, P. aphanidermatum* alone and a healthy control (no *Pythium*, no bacteria).

Seven and eleven days after transplant, another 100 ml of bacterial suspension (10^8 cfu/ml) were added to each plant. After two wk, 200 ml of a zoospore suspension (500 zoospores/ml) of isolate 186 of *P. aphanidermatum* were applied. Cucumbers were harvested, weighed, and measured daily when they had reached a diameter of 4.5 cm. Plant wilting and mortality were recorded. Plants were harvested after two months of fruit production. The trial began on 28 February 1994 with the first seeding of the rockwool cubes and ended in early June.

Statistical design and analysis

For the Summerland trial, data were analyzed by ANOVA and Duncan's means separation tests. Data in the Agassiz and Quebec trial were analyzed with SAS GLM model multivariate analysis of variance (MANOVA). MANOVA analyzes the data as vectors instead of individual observations, and permits hypothesis testing of the bacterial effect, while simultane-

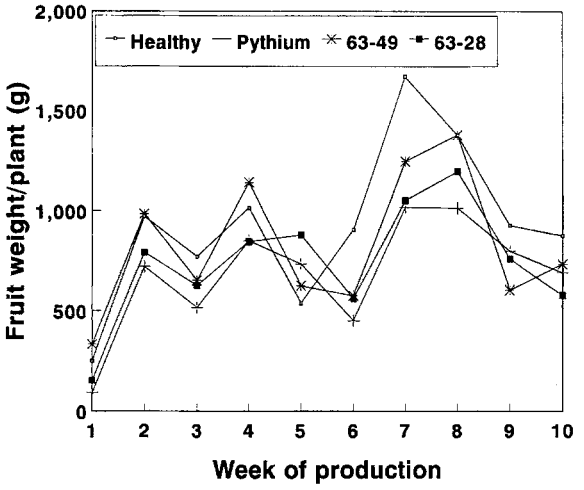

Figure 2. Effect of bacterial treatments and inoculation with *Pythium aphanidermatum* on weekly yield (weight of cucumbers) of plants grown in rockwool, Agassiz trial. Healthy: no bacterial treatment or *Pythium* inoculation; *Pythium*: no bacterial treatment, inoculated with *Pythium aphanidermatum*; 63-49: treated with strain 63-49 prior to *Pythium* inoculation; 63-28: treated with strain 63-28 prior to *Pythium* inoculation.

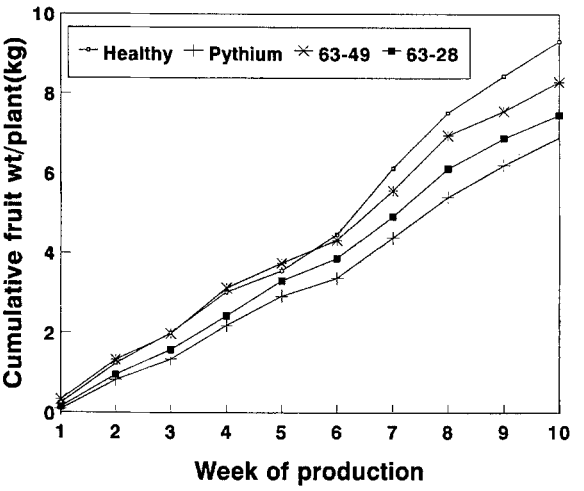

Figure 4. Effect of bacterial treatments and inoculation with *Pythium aphanidermatum* on cumulative yield (weight of cucumbers) of plants grown in rockwool, Agassiz trial. Healthy: no bacterial treatment or *Pythium* inoculation; *Pythium*: no bacterial treatment, inoculated with *Pythium aphanidermatum*; 63-49: treated with strain 63-49 prior to *Pythium* inoculation; 63-28: treated with strain 63-28 prior to *Pythium* inoculation.

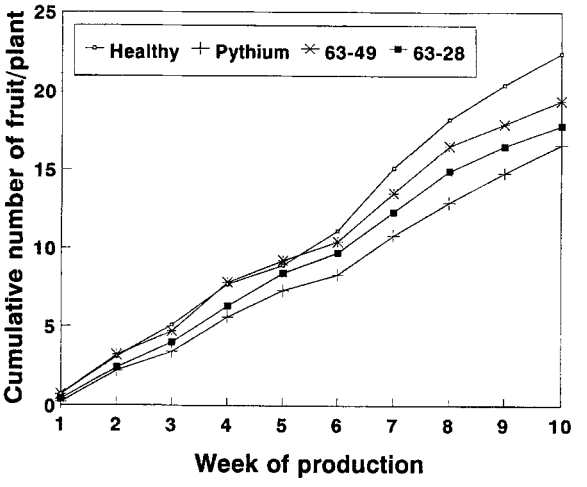

Figure 3. Effect of bacterial treatments and inoculation with *Pythium aphanidermatum* on cumulative yield (number of cucumbers) of plants grown in rockwool, Agassiz trial. Healthy: no bacterial treatment or *Pythium* inoculation; *Pythium*: no bacterial treatment, inoculated with *Pythium aphanidermatum*; 63-49: treated with strain 63-49 prior to *Pythium* inoculation; 63-28: treated with strain 63-28 prior to *Pythium* inoculation.

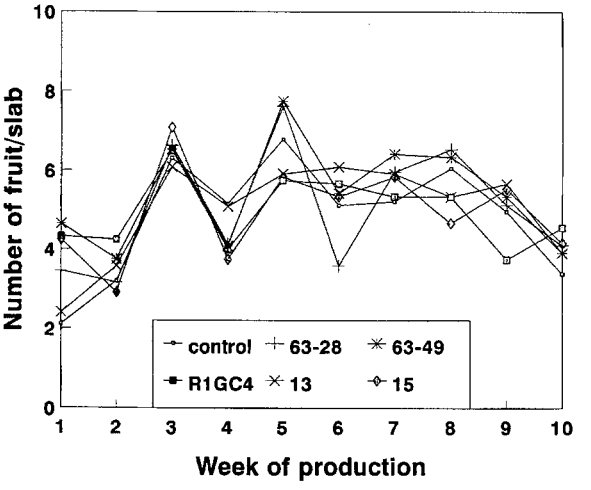

Figure 5. Effect of bacterial treatments and inoculation with *Pythium aphanidermatum* on weekly yield (number of cucumbers) of plants grown in rockwool, Quebec trial. Results from *Pythium* inoculated and non-inoculated treatments were pooled. Control: no bacterial treatment. Treatments with strain 63-49 produced significantly more cucumbers ($P = 0.05$) than the control at the 1-week harvest.

ously testing the bacteria X time interaction (Crowder and Hand, 1990). Main effects and time interactions were tested using the Wilks' Lambda statistic. Since the same individual plants were continually sampled over time, each time sample was not independent of the previous sample, so a repeated measures analysis of variance was also performed. A univariate test of hypothesis was performed using the Greenhouse Geisser Epsilon test. Individual treatments were contrasted with repeated measures ANOVA using a test of hypothesis between subject effects and an analysis of variance of contrast variables fitted to a linear or quadratic model. Because the Agassiz trial was not

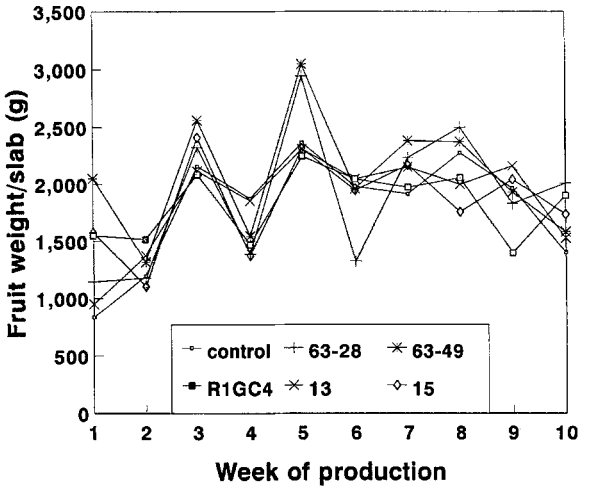

Figure 6. Effect of bacterial treatments and inoculation with *Pythium aphanidermatum* on weekly yield (weight of cucumbers) of plants grown in rockwool, Quebec trial. Results from *Pythium* inoculated and non-inoculated treatments were pooled. Control: no bacterial treatment. Treatments with strain 63-49 produced significantly more weight of cucumbers (P = 0.05) than the control at the 1-week harvest.

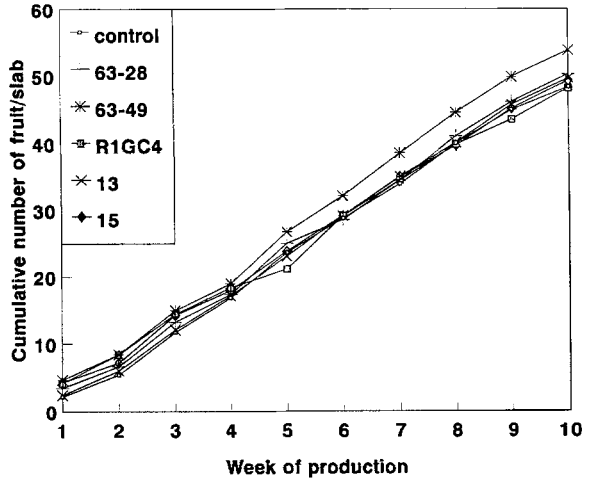

Figure 7. Effect of bacterial treatments and inoculation with *Pythium aphanidermatum* on cumulative yield (number of cucumbers) of plants grown in rockwool, Quebec trial. Results from *Pythium* inoculated and non-inoculated treatments were pooled. Control: no bacterial treatment.

blocked, experimental error due to location in the greenhouse was removed by including an X and Y plant location coordinate variable in the analyses.

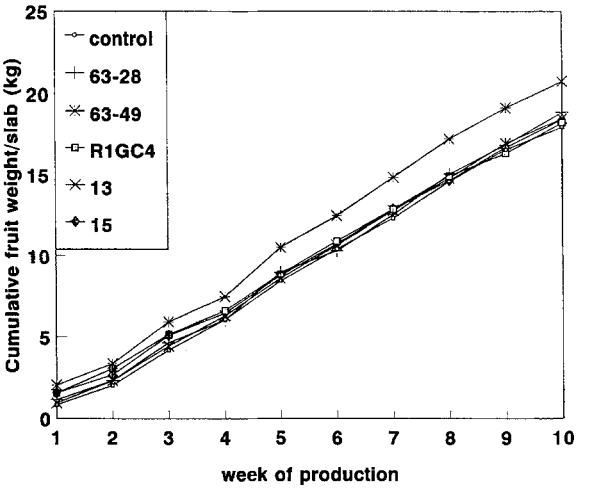

Figure 8. Effect of bacterial treatments and inoculation with *Pythium aphanidermatum* on cumulative yield (weight of cucumbers) of plants grown in rockwool, Quebec trial. Results from *Pythium* inoculated and non-inoculated treatments were pooled. Control: no bacterial treatment.

Results

British Columbia

In the Summerland trial, both strains 63-28 and 63-49 showed marked growth promoting ability in the first few days after seeding. The treated seeds germinated more rapidly, and reached the first true leaf stage 2 days before the positive and negative controls. By day 29, 11 days after the first bacterial drench, the negative effects of inoculation with *P. aphanidermatum* on plant height were evident, but not statistically significant until Day 42 (Table 1). Strains 63-49 and 63-28 reduced the detrimental effects of *Pythium*, up to day 42. Average dry weights of untreated plants and plants treated with 63-49 + *Pythium* were significantly higher (P = 0.05) than that of the *Pythium*-treated plants at day 42 (Table 2). There was no statistical difference in fruit yield among the treatments (data not shown).

Cucumber production in the Agassiz trial increased from week 1 to week 7, with yields and week-to-week fluctuations similar to those in the Quebec experiment (Figures 1 and 2). After week 6, the highest production of fruit numbers and weight were seen in the healthy non-inoculated plants, with the lowest yields in the *Pythium*-inoculated treatment without bacteria.

With classical ANOVA and MANOVA, there was no significant effect of plant location in the greenhouse or treatment, but the effect of time was significant. No variables interacted with time. With repeated measures

Table 1. Height of hydroponically grown cucumber plants seed-treated and drenched with *Pseudomonas fluorescens*, strains 63-28 and 63-49 and inoculated with *Pythium aphanidermatum* (Summerland, B.C. trial)

Treatment	Average plant height (cm)		
	Day 29	Day 42	Day 54
None	8.4 bc	99.3 a	201.7 a
63-28 + *Pythium*	9.5 ab	83 ab	203.1 a
63-49 + *Pythium*	10.7 a	82.2 ab	204.8 a
Pythium	6.8 c	75.3 b	187.5 a

Treatment means followed by the same letters are not significantly different according to Duncan's Mean Separation Test, $P = 0.05$.

Table 2. Average plant dry weight of hydroponically grown cucumber plants seed-treated and drenched with *Pseudomonas fluorescens* strains 63-28 and 63-49 and inoculated with *Pythium aphanidermatum* (Summerland, B.C. trial)

Treatment	Average plant dry weight	% of untreated control
None	89.2*	100
63-28 + *Pythium*	80.1	89.7
63-49 + *Pythium*	84.3*	94.5
Pythium	69.6	78

* Significantly different from plants treated with *Pythium* alone ($P = 0.05$).

analysis, the effect of treatment on cucumber weight and number was significant (P = 0.041 and 0.031, respectively). Treatments with *Pythium* alone or 63-28 had significantly less cucumber weights than the healthy control (P = 0.009 and 0.029, respectively). Similar significant contrasts were seen with number of fruit. Treatments with 63-49 were not significantly different from either the healthy control or the *Pythium*-inoculated treatment. The trends in yield over time did not fit a linear or quadratic model. The cumulative increase in cucumber number and weight over time showed a linear response.

Quebec. Due to the cold temperatures in April, 1994, the slab temperatures dropped below 20 °C at night, which was not favorable for the development of *P. aphanidermatum*. The effect of *Pythium* inoculation on cucumber yields (number and weight) was not significant in any of the analyses, although *Pythium* inoculum was detected by plating in the slabs throughout the experiment. No plant mortality was recorded, although some control plants were replaced after transplanting because of cold damage. Therefore, data were pooled between the treatments with and without *Pythium*. Treatment with 63-49 caused an early growth stimulation, which was reflected in significantly greater fruit numbers and weights during the first week of production, compared to the nontreated control plants (Figures 5 and 6). Fruit numbers and weight increased over the first 7 weeks, but fluctuated from week to week. Higher yields in week 3 and 5 were followed by lower yields in weeks 4 and 6. Heavy fruit set one week resulted in lighter fruit set the following week. The week-to-week fluctuation during the period 3–5 weeks was higher in plants treated with 63-49 and 63-28, compared to the non-treated control. After week 7, fruit production in general declined.

Using classical ANOVA, there was no effect of bacteria treatment or *Pythium* inoculation on cucumber numbers or weight. However, bacterial treatment had a significant effect on cucumber numbers (P = 0.059) and cucumber weight (P = 0.009), when analyzed with MANOVA. Time also had a significant effect, but not *Pythium* inoculation. When analyzed with a repeated measures ANOVA, time had a significant effect on both cucumber number and weight, and interacted with bacterial treatment (P = 0.0511 and 0.045, respectively). Using repeated measures analysis tests for between subject effects, plants treated with 63-49 had more fruit number (P = 0.045) and weight (P = 0.068) compared to nontreated control plants. None of the other treatment contrasts were significant. Both cucumber number and weight showed a significant quadratic response over time. The response of fruit number and weight over time in plants treated with R1GC4 and 15 showed a flatter quadratic trend that was significantly different from the untreated control plants.

Cumulative numbers and weights of cucumbers were plotted over time (Figures 7 and 8). The increase in production for numbers and weights showed a significant linear response (R^2 = 0.91 – 0.93). The increase in cucumber weight and number over time (slope) was significantly greater in the 63-49 treatment, compared to all other treatments. The slopes of all bacteria treatments except 63-49 were not significantly different from the control, for both number and weight. For cucumber weight, the Y-intercepts of the R1GC4 and 15 treatments were significantly greater than the control treatment. For cucumber number, the regressions for treatments R1GC4, 13, and 15 had higher Y-intercepts than the control treatment. By the end of the experiment, slabs treated with 63-49 yielded six

more fruit and 3 kg more weight, an increase of 12% and 18%, respectively.

Discussion

Besides the fact that there are no fungicides currently registered against zoosporic pathogens for use in soilless culture in the United States or Canada, there are many potential advantages to the use of biocontrol agents in these systems. The environmental conditions are uniform and can be controlled to favor the biocontrol agent and inhibit the pathogen. There is a lack of competition from soil microflora in soilless substrates during the early stages of the crop, which should favor the establishment of biological control agents. The inoculum of biocontrol agents can be easily introduced into the fertigation or irrigation system, and the high value of glasshouse crops would justify the higher cost of biocontrol agents. Biocontrol agents are considered to have less environmental impacts than fungicides. Despite these advantages, very little work has been done on developing biocontrol agents for zoosporic pathogens in soilless systems, and few studies have examined the effect of biocontrol agents on mature vegetables. Paulitz et al. (1992) selected rhizosphere bacteria for the control of *Pythium aphanidermatum* on cucumber. When tested under near-commercial conditions in rockwool inoculated with *P. aphanidermatum*, isolates of *P. corrugata* (strain 13) and *P. fluorescens* (strain 15) significantly reduced disease under high disease levels, but did not increase yields to the level of the healthy controls (Rankin and Paulitz, 1994). Both strains 13 and 15 increased yields in the absence of the pathogen, suggesting a plant growth-promoting (PGPR) effect. Moulin et al. (1994a) found one strain of *Pseudomonas* spp. that controlled *P. aphanidermatum* under near-commercial conditions, resulting in yields comparable to the uninoculated control. However, the disease level was low, since the pathogen alone, without the biocontrol agent, only reduced the yields by 17%. *P. fluorescens* strain WCS365 and the commercial product Mycostop (*Streptomyces griseo-viridis*) reduced disease caused by *P. aphanidermatum* on cucumbers by 30–50% in an ebb and flow hydroponic system in the Netherlands (Postma et al., 1995). *Bacillus subtilis* has also been found to reduce *Phytophthora nicotianae* var. *nicotianae* on tomatoes (Bochow, 1992) and the mycoparasite *Pythium oligandrum* antagonized *Pythium splendens* on cucumber (Thinggaard et al., 1988).

In this study, we tested a range of bacterial isolates from different development programs under near-commercial conditions in three trials and locations, and measured the yields on a daily basis for the entire production period. A number of reproducible trends were evident. Strain 63-49 performed the best of all strains tested, and had a growth-promoting effect in the absence of measurable disease pressure or *Pythium* inoculation (Quebec trial). This strain, originally isolated from a canola field in Winnipeg, Canada (Kloepper et al., 1988), improved cucumber yields 12–18%. This is comparable to the growth promotion seen by other PGPR bacteria in greenhouse crops. More important, this strain significantly increased cucumber yields of the first harvests, which may be more economically beneficial to the grower than yield increases later in the season. Strain 63-49 was among the higher ranked of 4000 bacterial strains tested for PGPR activity, and increased canola yield by 15% under field conditions (Kloepper et al., 1988). This strain did not demonstrate in vitro antibiosis against *Pythium ultimum*, but did have some activity against *Rhizoctonia solani* (Reddy et al., 1993). PGPR strains have been shown to increase yields in many other crops, including peanut (Turner and Backman 1989), wheat (de Freitas and Germida 1989), cotton (Backman et al., 1994), and container-grown plants (Harris, 1994). The mechanisms by which this is achieved could be due to indirect effects on the plant by directly antagonizing pathogens via siderophore, antibiotic, or hydrogen cyanide production (Kloepper et al., 1991). PGPR have also been shown to induce resistance to root and foliar pathogens (Liu et al., 1995; Zhou and Paulitz, 1995). PGPR may also inhibit deleterious rhizobacteria, or directly stimulate plant growth through the production of plant hormones (Arshad and Frankenberger, 1991) or increased phosphorus uptake.

Strain 63-28 was shown to increase the yield of tomatoes 13–18%, but only under suboptimal fall conditions (Gagné et al., 1993). This strain produces several potent antifungal compounds that are effective against *Pythium ultimum* and *Phytophthora cryptogea* in vitro (Gamard et al., 1995). This strain had no significant effect on weekly cucumber yield in the absence of disease pressure (Quebec trial), but did significantly increase the cumulative number of cucumbers over time, compared to the *Pythium*-inoculated plants without biocontrol treatment. Strains 13 and 15 showed a slight degree of growth promotion, but less than reported by Rankin and Paulitz (1994). The slab temperatures were much cooler in the trial reported

here, especially during the early part of the season, and these bacteria may not be effective under this temperature regime. Strain R1GC4 showed similar effects to strain 13 and 15.

During weeks 3–5, fruit production fluctuated from week to week. The fruit production in treatments with strains 63-49 and 63-28 fluctuated even more than the control treatment in the Quebec trial. Whatever the mechanism of increased fruit production, a heavy fruit set induced by the PGPR bacteria was followed by a fruit set lighter than the control the following week. Despite this fluctuation, the cumulative yields over time were not reduced.

The demonstrated increase in yield in the absence of measurable disease (Quebec) or under moderate disease pressure (Agassiz) could offer growers additional advantages over a strain that only acts as a biocontrol agent. In addition, these strains could have a prophylactic effect, if added at multiple times over the course of the season. Most of the previous research has evaluated the biocontrol agents added to the system 1–3 times a few weeks prior to inoculation with high populations of the pathogen. In a commercial situation, however, the pathogen is probably introduced at multiple times over the season, beginning early in the season, in a random fashion at low inoculum densities. Future tests of the strains should be made under these more realistic inoculum loads. Under these conditions, *Pseudomonas* spp. may prevent the establishment of *Pythium* spp. or delay the build-up of secondary inoculum and the spread to surrounding roots and plants. The main goal would be to keep the inoculum density below the economic threshold. Menzies et al. (1996) showed that inoculum densities as low as 22 zoospores of *P. aphanidermatum* added to 100 L of nutrient solution in nutrient film culture significantly reduced the yield of cucumbers.

In conclusion, the use of biocontrol or PGPR bacteria has the potential to be a useful component of an integrated disease management strategy for cucumbers grown in soilless systems.

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