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Interactions between fluorescent pseudomonads and VA mycorrhizal fungi

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SUMMARY

Cucumber seeds were treated with rifampin-resistant derivatives of *Pseudomonas putida* (A12, N1R or R-20) or *P. fluorescens* (2-79 or 3871) and planted in soils with and without added inoculum of the VA mycorrhizal fungi *Glomus intraradices* Schenck & Smith or *G. etunicatum* Becker & Gerdemann. Populations of *Pseudomonas* spp. in the combined rhizosphere-rhizoplane soil were determined at 1–9 weeks after planting by dilution plating on a selective medium. At 1–3 weeks, the populations of all strains except R-20 were 1·5 to 7 times lower in the rhizosphere of cucumber roots colonized by *G. intraradices*, when compared to nonmycorrhizal plants. However, this effect was less consistent in 3- to 9-week-old plants. No significant difference was detected in populations of *Pseudomonas* strains between roots colonized by *G. etunicatum* and nonmycorrhizal roots. Strains 3871 and 2-79, which are antibiotic producers, delayed the germination of *G. etunicatum* spores in raw soil, but by seven days no significant differences in frequency of germination were detected. None of the fluorescent *Pseudomonas* strains affected the colonization of cucumber roots by *G. etunicatum*, as determined by measurements of mycorrhizal inoculum density–root colonization relationships.

Key words: Vesicular–arbuscular mycorrhizal fungi, fluorescent pseudomonads, cucumber, *Cucumis sativus*, *Pseudomonas putida*, *P. fluorescens*, *Glomus intraradices*, *G. etunicatum*, mycorrhizosphere.

INTRODUCTION

Fluorescent *Pseudomonas* spp. have been implicated in the biological control of numerous plant diseases, including *Rhizoctonia* and *Pythium* damping-off of cotton (Howell & Stipanovic, 1979, 1980), *Fusarium* wilt of cucumber (Scher & Baker, 1982), and take-all of wheat (Weller & Cook, 1983). In the iron-limiting environment of the rhizosphere, fluorescent pseudomonads produce siderophores, which are secondary metabolites with a high affinity for Fe^{3+}. Competition for iron between *Pseudomonas* spp. and plant pathogens has been proposed as one mechanism of biological control (Scher & Baker, 1982; Kloepper *et al.*, 1980*a*, *b*). Some fluorescent pseudomonads also produce anti-fungal compounds (Howell & Stipanovic, 1979, 1980; Gurusiddaiah *et al.*, 1986). Many fluorescent pseudomonads are also considered to be plant growth-promoting rhizobacteria (PGPR), and can stimulate plant growth independent of any major pathogen involvement (Kloepper *et al.*, 1980*a*, *b*; Suslow & Schroth, 1982).

Very few studies have examined the possible interactions between biocontrol/plant growth-promoting agents and vesicular-arbuscular (VA) mycorrhizal fungi. These mycorrhizal fungi are a ubiquitous component of most agroecosystems, and VA mycorrhizas provide numerous benefits to their host, including better phosphorus nutrition (Tinker, 1984), increased drought tolerance (Nelsen, 1987), increased disease resistance (Caron, Fortin & Richard, 1986), and enhancement of nitrogen fixation from rhizobia (Subba Rao, Tilak & Singh, 1986; Kawai & Yamamoto, 1986). If biological control agents are to be widely used in horticultural or agricultural systems, they must be compatible with mycorrhizal fungi. Biocontrol agents such as fluorescent pseudomonads might antagonize not only fungal plant pathogens but also mycorrhizal fungi, through the production of anti-fungal compounds or siderophores. Mycorrhizal fungi also exert profound effects on other rhizosphere microorganisms, either through indirect effects on host physiology and changes in root exudation or through direct effects via fungal exudates. This concept, called the mycorrhizosphere effect, is reviewed by Linderman (1988) and Paulitz & Linderman (1990). These qualitative and quantitative changes in rhizosphere microflora might stimulate or inhibit the colonization and establishment of introduced bacterial biocontrol

agents such as fluorescent pseudomonads. Biocontrol agents might also stimulate mycorrhizal infection, as demonstrated by Meyer & Linderman (1986*b*). The use of biological control agents that have negative interactions with mycorrhizal fungi would be self-defeating in terms of plant productivity, and these interactions must be identified and characterized. Such an interaction was reported by Krishna, Balakrishna & Bagyaraj (1982), who observed antagonism between *Streptomyces cinnamomeous* and *Glomus fasciculatum*.

The purpose of this paper was to examine (1) the effect of VA mycorrhizal fungi on colonization of cucumber roots by *Pseudomonas* spp. applied to the seed and (2) the influence of *Pseudomonas* spp. on germination of VA mycorrhizal fungal spores and subsequent colonization of cucumber roots by VA mycorrhizal fungi. A brief report of the present study has been published (Paulitz & Linderman, 1989).

MATERIALS AND METHODS

Soil

A 1:1 mixture of Willamette sandy loam soil and river sand was used for all experiments. The soil, pH 6·0, contained 0·02 % total nitrogen, 9 mg kg^{-1} phosphorus, 82 mg kg^{-1} potassium, and 8·7 mequiv. calcium per 100 g of soil. The soil-sand mix was pasteurized at 70 °C for 45 min, to eliminate the native species of VA mycorrhizal fungi. To re-establish the native microflora, a filtrate from the non-pasteurized soil was prepared by mixing 200 g soil with 2 l of water. The soil–water mixture was filtered through a 38 μm sieve to remove spores of VA mycorrhizal fungi, and was mixed with 40 l of pasteurized soil. The filtrate-pasteurized soil mix was incubated in open containers in the greenhouse for 1 month, and was periodically moistened.

Biological materials

The following strains of *Pseudomonas putida* and *P. fluorescens* were used – *P. putida* strains A-12 (Park, Paulitz & Baker, 1988), N1R (Dupler & Baker, 1984), and R-20 (Meyer & Linderman, 1986*a*); and *P. fluorescens* strain 3871 (Loper, unpublished) and 2-79RN_{10} (Weller & Cook, 1983). All strains were spontaneous mutants that were selected for resistance to rifampin. Strain 3871 was a rifampin-resistant mutant derived from strain PF5 (Howell & Stipanovic, 1980). The bacteria were initially cultured on nutrient agar, and were stored at −80 °C in nutrient broth amended with 10 % glycerol. For each experiment, the bacteria were retrieved from cold storage and streaked on to nutrient agar.

Two species of VA mycorrhical fungi were used-*Glomus intraradices* Schenck & Smith and *G. etunicatum* Becker & Gerdemann. The mycorrhizal inoculum, a mixture of sand, roots, and spores, was obtained from Native Plants Inc., Salt Lake City, Utah. The concentration of spores in the inoculum was determined by wet-sieving the inoculum through a 500 μm mesh, and collecting the filtrate on a 38 μm mesh. Spores, roots, and organic matter were decanted off, and spores were counted with a dissecting microscope set at 30 ×. The inoculum was diluted with soil in which the native microflora was re-established by mixing in a twin-shell blender, to obtain the desired spore concentration.

Bacterial seed treatments

Bacteria were grown on King's B agar (King, Ward & Raney, 1954) for 3 d at 27 °C. Ten ml of sterile distilled water were poured on each culture plate, and the bacteria were scraped from the plate with a sterile glass rod. The bacterial suspension was centrifuged at 2000 ***g*** for 15 min, the supernatant was discarded, and the pellet was re-suspended in 0·1 M $MgSO_4.7H_2O$. The suspension was centrifuged and re-suspended two more times. The optical density of the bacterial suspension was measured at 640 nm on a spectrophotometer, and was adjusted to 0·5 absorbance units, which corresponded to approximately 1×10^9 cells ml^{-1}. Pelgel, a seed sticker (The Nitragen Co., Milwaukee, WI) was mixed with the bacterial suspension (2 %, w/v), and cucumber seeds (*Cucumis sativus* L. 'Marketer Long') were soaked in the bacterial suspension for 1 h prior to planting. In preliminary experiments, dilution plating showed that each seed was coated with $1{\cdot}38–4{\cdot}30 \times 10^8$ bacteria per seed.

Bacterial soil treatments

Bacteria were grown in King's B broth (50 ml in 125 ml Erlenmyer flasks) on a rotary shaker for 3 d at 27 °C. The bacterial broth was filtered through three layers of sterile cheesecloth, centrifuged, and re-suspended as described previously. The bacterial suspension was adjusted to 0·5 absorbance units, added to distilled water (10 %, v/v), and mixed by hand into soil (10 ml bacterial suspension/100 g soil). This resulted in approximately 1×10^7 bacteria g^{-1} soil, which was confirmed by dilution plating in preliminary experiments.

Effect of VA mycorrhizal fungi on fluorescent pseudomonads

To test the effect of VA mycorrhizal fungi on rhizosphere colonization by fluorescent pseudomonad strains, cucumber seeds were coated with bacteria and planted in soil with or without *Glomus intraradices* or *G. etunicatum*. In the mycorrhizal soil treatments, *G. intraradices* was added at 11·3 spores g^{-1} soil, and *G. etunicatum* was added at 18·6 spores

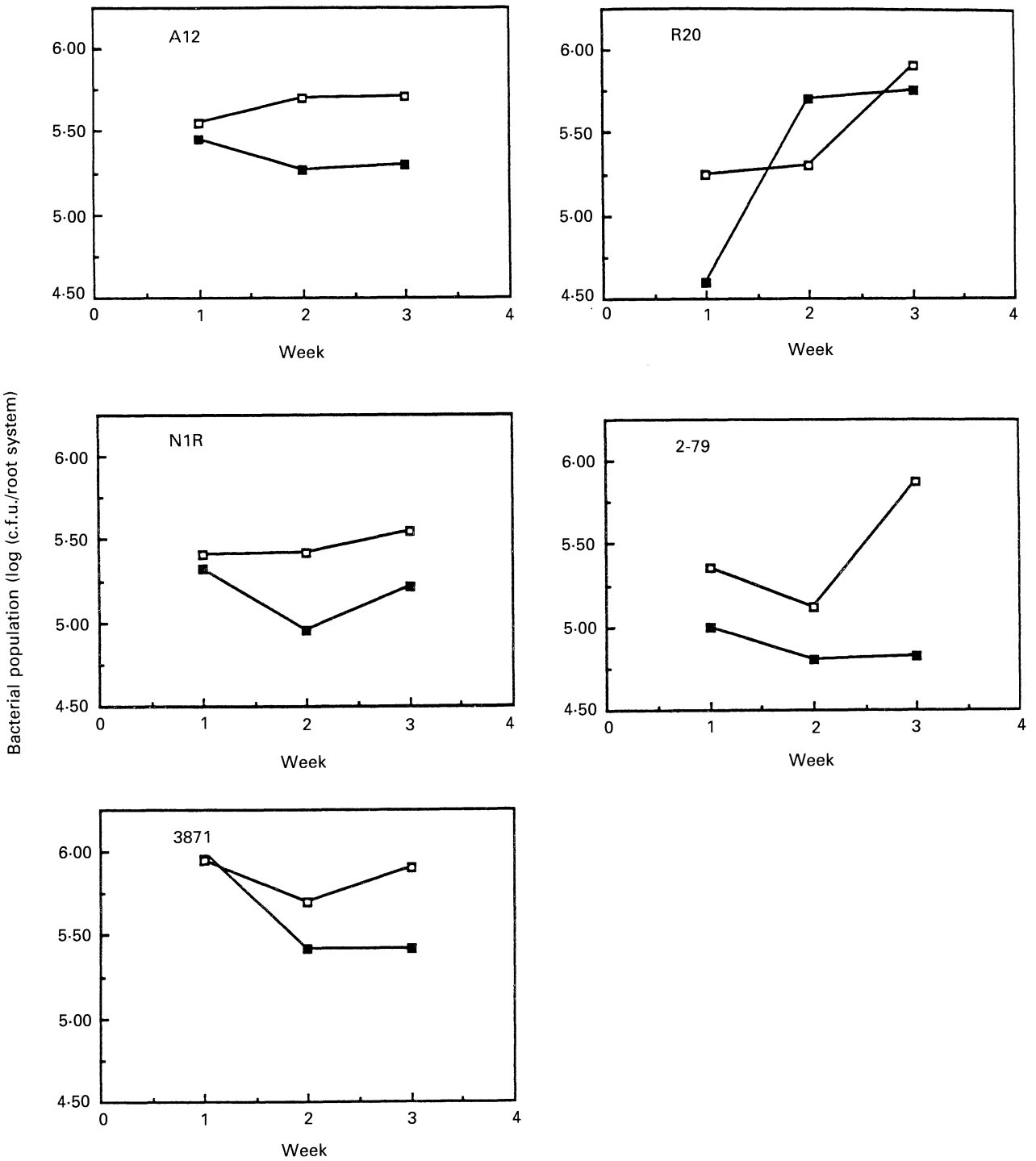
g^{-1} soil. The soil was placed in 21 × 4 cm diameter plastic tubes (Leach Cone-Tainer Co., Canby, OR) that were plugged on one end with a rayon ball. One seed was planted in each tube, and each treatment had 30 replicate tubes. A water suspension from the mycorrhizal inoculum was filtered through a 38 μm sieve, and 10 ml were added to each tube. The plants were grown in a greenhouse in controlled-root temperature boxes, which were maintained at 26 °C. Lighting was supplemented with high-pressure sodium-vapour lamps (350–480 $\mu E\ m^{-2}\ s^{-1}$, 16 h d^{-1}). Plants were watered daily, and fertilized once a week with a modified Long Ashton nutrient solution with $\frac{1}{4}$ strength P (Hewitt, 1966) At 1, 2 and 3 weeks after planting, eight plants were harvested from each treatment in the experiment with *G. intraradices*. In the experiment with *G. etunicatum*, plants were sampled at 2, 3 and 4 weeks after planting. Loosely adhering soil was shaken from the roots, and the upper 1 cm of root closest to the seed was removed. The remaining root system was placed in a flask containing 99 ml of sterile distilled water, and the flasks were sonicated in an ultrasonic cleaner (Sonix IV, Inglewood, CA 90302 USA) for 3 min. Sonicated roots were removed, cleared, and stained according to the method of Phillips & Hayman (1970) to determine mycorrhizal colonization. The suspension was dilution-plated on King's B agar amended with rifampin at 100 mg l^{-1} and cycloheximide at 100 mg l^{-1}. Bacterial numbers were log-transformed and averaged for each treatment. Rhizosphere populations were expressed as mean log number of bacteria per root system. In preliminary experiments, populations were calculated on the basis of the dry weight of rhizosphere soil, as well as per root system. The coefficient of variation was not reduced when the values were expressed on a per weight basis, and there was no significant difference in root dry weight or rhizosphere soil dry weight among the treatments.

To examine long-term effects, similar experiments with *G. intraradices* were performed in 12 × 12 cm round pots (1 kg soil per pot), with eight replicate pots per treatment. Two-hundred g of inoculum of *G. intraradices* (11·3 spores g^{-1}) were placed in a 12 × 4 cm diameter cylindrical hole in the centre of the pot. Three seeds treated with bacteria were planted on top of the inoculum cylinder, and were thinned to one plant after 1 week. Plants were grown in the greenhouse (22–28 °C), fertilized as previously described, and light was supplemented with high pressure sodium vapour lighting (250 $\mu E\ m^{-2}\ s^{-1}$, 16 h d^{-1}). Plants were arranged on the greenhouse bench in a completely randomized design. At 3, 6, and 9 weeks after planting, a soil-root sample was removed from each pot 1 cm from the stem with a no. 8 core borer. Adhering soil was removed from the roots, which were processed as described previously. The hole was filled with re-colonized soil, and subsequent samples were removed at an angle of 90° from the previous sample, relative to the stem.

Effect of fluorescent pseudomonads on a VA mycorrhizal fungus

To examine the effects of fluorescent pseudomonads on the germination of spores of VA mycorrhizal fungi, the following experiment was performed. Spores of *G. etunicatum* were removed from the fungal inoculum by wet sieving and decanting as described previously. Bacterial inoculum was prepared according to the methods described in 'bacterial seed treatments'. Approximately 300 spores of VA mycorrhizal fungi were vacuum-filtered onto a 47 mm diameter cellulose triacetate filter (0·2 μm pores). One ml of bacterial suspension, containing approximately 10^8 bacteria ml^{-1}, was also vacuum-filtered onto the membrane. Another membrane was placed on top, and the spore-membrane sandwich was buried in 40 g of raw soil-sand in a 100 × 15 mm plastic Petri dish. The soil was moistened with distilled water (15 %, w/w). The dishes (8 replicates per treatment) were incubated at 27 °C. At 4, 5, 6, 7 and 8 days, a pie-shaped wedge representing one-eighth of the membrane sandwich was cut and removed from each dish. The sandwich was opened, the adhering soil was removed from the bottom surfaces, and 2–3 drops of 0·05 % Trypan blue was placed on each membrane to stain the fungal germ tubes. The stained sections of membrane were examined at 200 × under a compound microscope. The number of germinated spores was expressed as a percentage of the total number, and a mean % germination was derived for each treatment.

The effects of fluorescent pseudomonads on mycorrhizal colonization was determined by comparing the inoculum density-root colonization relationships in treatments with and without the bacterial strains. Six inoculum densities of *G. etunicatum* were prepared by diluting sand-root inoculum with re-colonized soil. The inoculum densities used were 18·60, 9·30, 4·65, 2·32, 1·16, and 0·58 spores g^{-1} soil. Bacteria were cultured and applied to the soil, as described in 'bacterial soil treatments'. Soil from each treatment was placed in 2.5 cm diameter × 16 cm plastic tubes (Leach Cone-Tainer Co., Canby, OR) (70 g per tube, 10 tubes per bacteria treatment-inoculum density). The tubes were placed in the greenhouse in controlled temperature boxes (26 °C). The soil was moistened daily, and incubated for one week before being planted with one seed per tube. After 12 days, the plants were removed from the tubes, the roots were washed, and placed in distilled water. The roots were cleared and stained according to the methods of Phillips & Hayman (1970). The percent mycorrhizal colonization was determined with the line intersect method, and mean colonization was calculated for



Figures 1 and **2.** The effect of *Glomus intraradices* on the population of fluorescent pseudomonad strains A12, N1R, 3871, R20, and 2-79 in the rhizosphere-rhizoplane of cucumbers. Bacteria were applied to the seed at approximately 10^8 bacteria per seed. The mycorrhizal fungus (■) was added to soil at 11·3 spores g^{-1}; (□), absence of VA mycorrhizal fungi. Pooled L.S.D. (95 % level) for Figs 1 and 2 were 0·33 and 0·41, respectively.

each bacteria treatment-inoculum dilution. A linear regression was performed on the root colonization and inoculum density data.

Statistical analyses

All experiments were made twice. An F test on the error mean square values of each strain treatment from the first and second trial showed that the trials were similar. Only the results from the first trial are presented. The *Pseudomonas* colonization experiments were designed as $5 \times 3 \times 2$ factorial experiments and analyzed using a general linear model procedure of SAS (SAS Institute, Cary, NC). Data from the mycorrhizal and nonmycorrhizal treatments of each strain were compared at each sampling

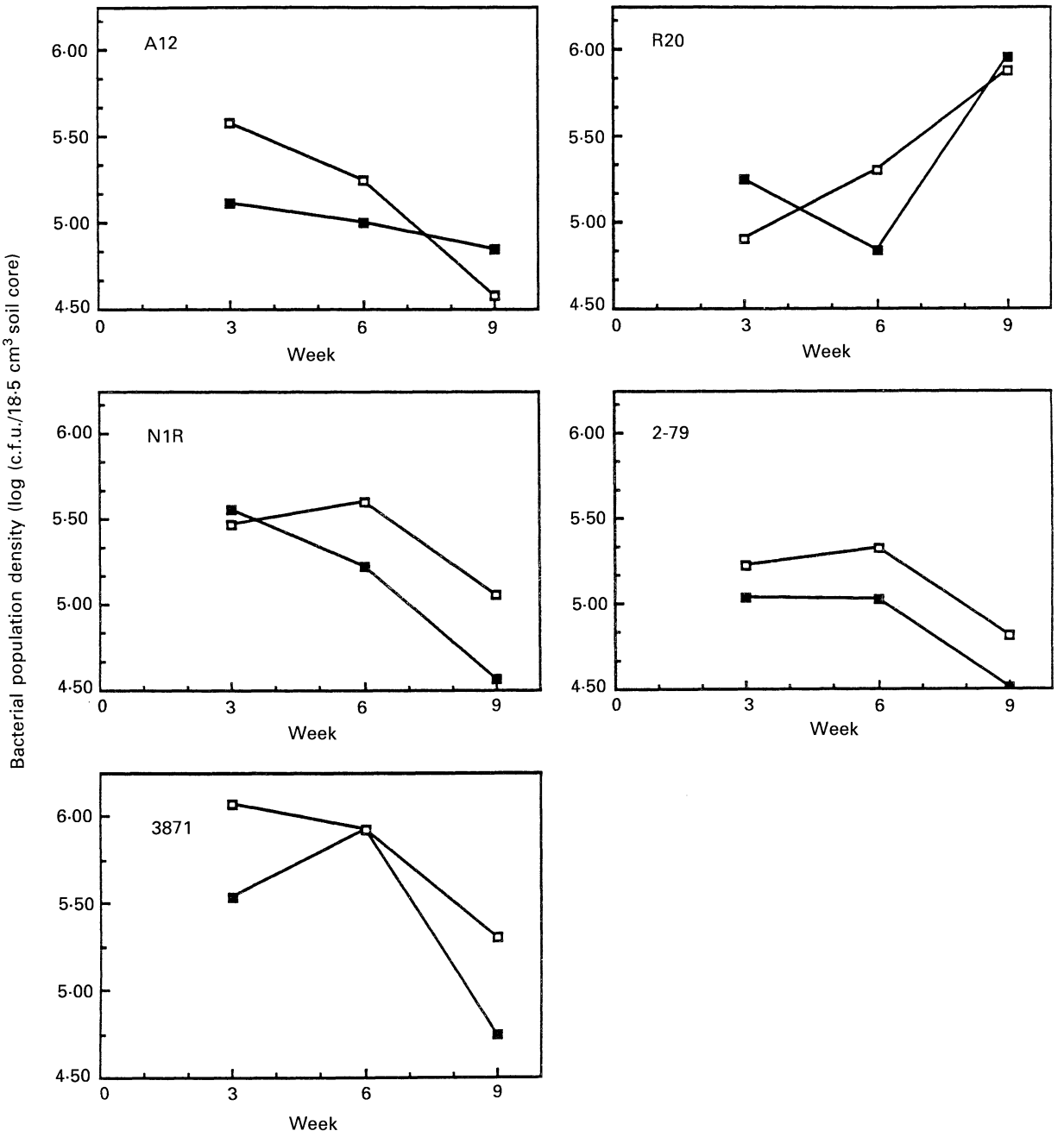


Figure 2. For legend see opposite.

time using a *t*-test. Data from spore germination experiments were analysed with a one-way analysis of variance at each sampling time. Linear regression models were constructed from the inoculum density–root colonization data. Statistical differences in the slope parameter between regressions were determined according to the methods of Steel & Torrie (1980).

RESULTS

Effect of VA mycorrhizal fungi on fluorescent pseudomonads

The population of fluorescent pseudomonads were significantly lower ($P < 0{\cdot}001$) in the rhizosphere of cucumber plants colonized by *Glomus intraradices*, compared to non-mycorrhizal plants, except for strain R-20, which had similar populations in both treatments (Fig. 1). Differences between the mycorrhizal and non-mycorrhizal treatments were most pronounced at the 3-week sampling date, where 1·5–7 times more bacteria were detected in the non-mycorrhizal treatment. The populations in both treatments were relatively constant over the 3–7 week period of the experiment, and in general did not vary more than 0·5 log units. The interaction between *G. intraradices* and *Pseudomonas* strains was not significant.

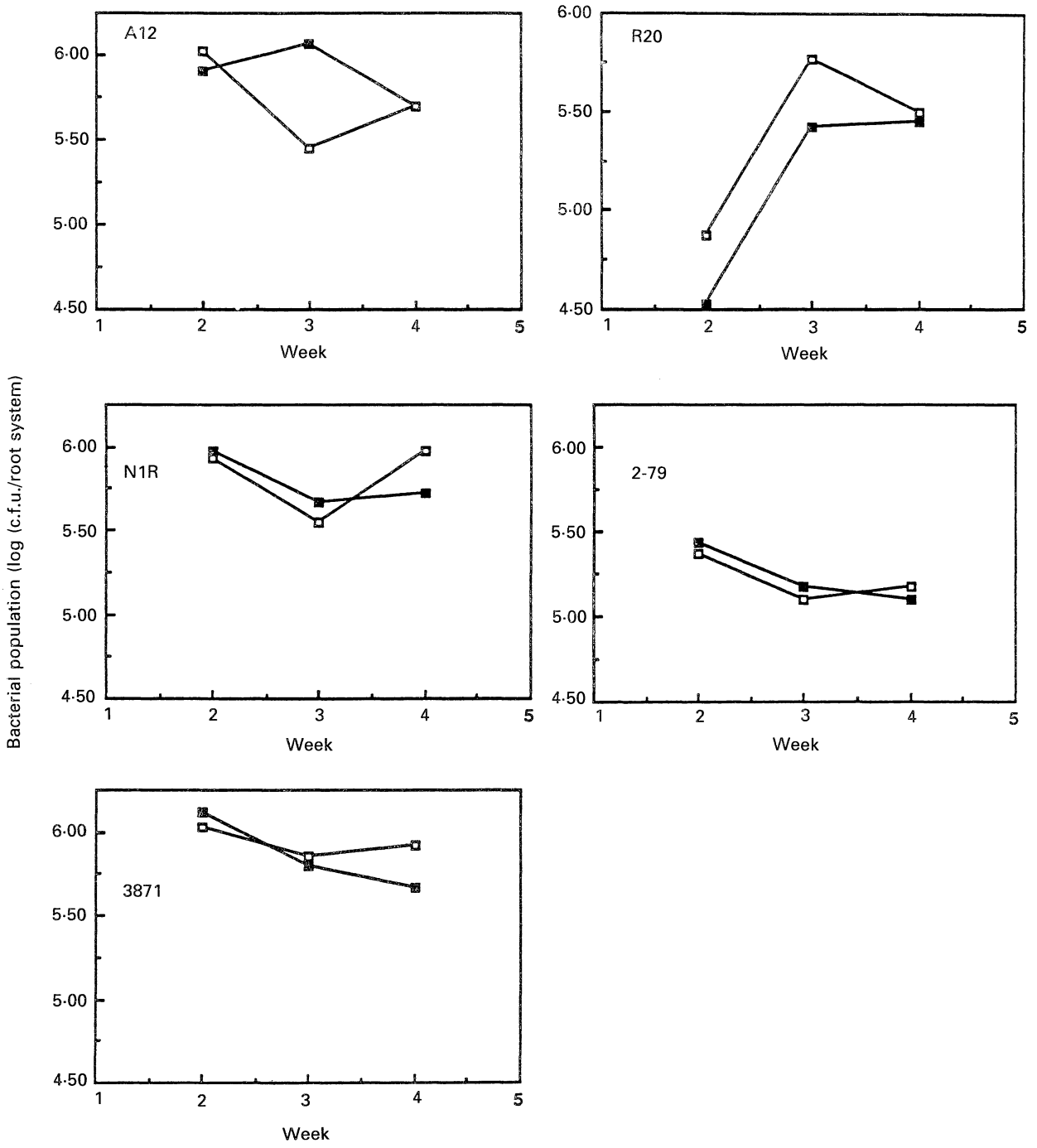


Figure 3. The effect of *Glomus etunicatum* on the population of strains of fluorescent pseudomonads in the rhizosphere-rhizoplane of cucumber. Bacteria were applied to the seed at approximately 10^8 bacteria per seed. The mycorrhizal fungus (■) was added to soil at 18·6 spores g^{-1}; (□), absence of VA mycorrhizal fungi. Pooled L.S.D. (95% level) for Figure 3 was 0·30.

In the long-term experiment, where populations were sampled at 3, 6 and 9 weeks after planting (Fig. 2), the populations of all *Pseudomonas* strains except R-20 and A12 were generally lower on cucumber roots colonized by *G. intraradices*, when compared to non-mycorrhizal roots. However, when the experiment was repeated, significant reductions were observed only in the treatments with strain N1R. In general, the populations of the bacteria in both the mycorrhizal and non-mycorrhizal treatments declined one long unit over the course of the experiment.

No significant differences were detected in the populations of *Pseudomonas* spp. between cucumber roots colonized by *Glomus etunicatum* and non-mycorrhizal roots (Fig. 3).

No significant difference in mycorrhizal colonization was detected between the *Pseudomonas* treat-

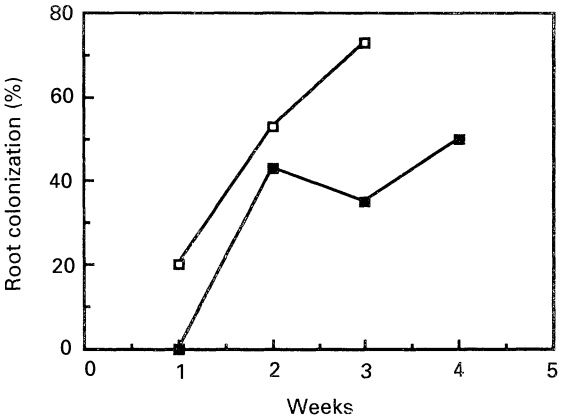


Figure 4. Colonization of cucumber roots by *Glomus intraradices* (□) and *G. etunicatum* (■) over time. *G. intraradices* and *G. etunicatum* were applied at 11·3 and 18·6 spores g^{-1}, respectively.

ments, so the data at each sampling time were pooled (Fig. 4). *G. intraradices* rapidly colonized cucumber roots, reaching 72 % after 3 weeks, and 91 % after 9 weeks. *G. etunicatum* was a slower colonizer, and colonization was not detected until 2 weeks after planting. With *G. intraradices*, a large proportion of the root tissue contained fungal biomass, and heavily colonized roots were filled with vesicles and internal chlamydospores after 3 weeks. Infections of *G. etunicatum* were more arbuscular, and fewer vesicles were formed in the root, when compared to *G. intraradices*. In experiments with *G. intraradices*, significant amounts of external hyphae in non-sonicated roots were not observed until the cucumber plants were 9 weeks old.

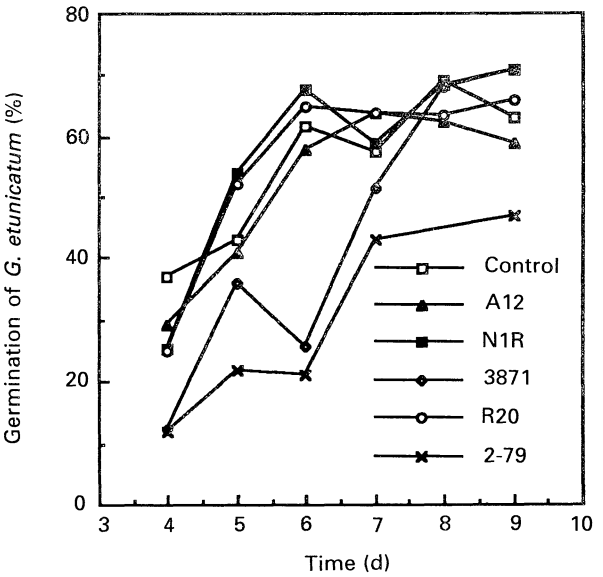


Figure 5. Effect of strains of fluorescent pseudomonads on germination of spores of *Glomus etunicatum*. Spores and bacteria were applied to 47 mm diameter cellulose triacetate filters (0·2 μm pore size), and buried in raw soil. Pooled L.S.D. (95 % level) was 13·3.

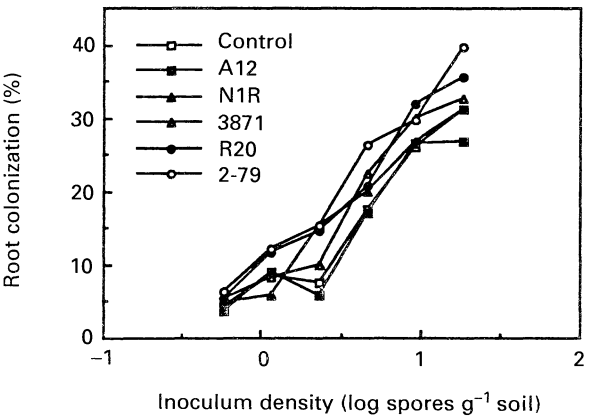


Figure 6. Effect of strains of fluorescent pseudomonads on VA mycorrhizal inoculum density–root colonization relationships of *Glomus etunicatum* on cucumber. Bacteria were applied at approximately 10^7 bacteria g^{-1} soil to soil containing different inoculum densities of the mycorrhizal fungus. Soil with added bacteria was moistened and incubated for one week prior to being planted with cucumbers. Twelve-day-old plants were harvested, and mycorrhizal colonization was determined. There were no significant differences in mycorrhizal root colonization among the bacterial strains tested.

Effect of fluorescent pseudomonads on a VAM fungus

Pseudomonas strains 3871 and 2-79 both delayed the germination of spores of *G. etunicatum* (Fig. 5). From day 4 to day 6, the spore germination in these treatments was significantly lower than the control. However, by day 7, the spore germination was similar in all treatments.

The inoculum density–root colonization relationships were similar in all *Pseudomonas* treatments (Fig. 6), as determined by a pairwise comparison of the slope parameter of regression. There was a linear relationship between percent mycorrhizal colonization at 12 days and initial log inoculum density of the mycorrhizal fungus. The percent of roots colonized by *G. etunicatum* ranged from 26–39 % at 18·65 spores g^{-1} to 3–7 % at 0·58 spores g^{-1}.

DISCUSSION

Glomus intraradices rapidly colonized cucumber roots, and reduced the rhizosphere populations of fluorescent pseudomonads that were introduced as a seed treatment. This mycorrhizosphere effect was most pronounced in cucumber seedlings that were 1–3 weeks old. At this early stage, the VA mycorrhizal fungus might present a significant carbon sink at a time when the photosynthetic source capacity of the host is low. Growth depressions observed in VA mycorrhizal plants at the early stages of symbiosis have been attributed to carbon competition. (Buwalda & Goh, 1982, Bethlenfalvay, Brown & Pacovsky, 1982). Autoradiographic studies have shown that large amounts of host carbon are

accumulated in fungal vesicles (Cox *et al.*, 1975). By pulse-chase labelling soybean plants with $^{14}CO_2$, Harris, Pacovsky & Paul (1985) estimated that 17% of the total photosynthate in 6-week-old soybean plants was allocated to the VA mycorrhizal symbiont. Large amounts of host carbon are also released by the root, by exudation and by sloughed-off cells. Barber & Lynch (1977), using $^{14}CO_2$, estimated that 20% of the fixed carbon is exuded by the root, most of which is metabolized by soil microbes and released as respiratory CO_2. A quantitative or qualitative change in allocation of carbon to the rhizosphere, mediated by heavy mycorrhizal colonization, might explain the small but significant reduction in populations of fluorescent pseudomonads, which are also dependent on host carbon. Infection by VA mycorrhizal fungi usually increases photosynthesis (Pang & Paul, 1980), probably because of increased uptake of phosphorus by the external hyphae. However, in our experiments, significant amounts of external hyphae were not produced until cucumber plants were 7–9 weeks old. Although we did not observe any growth depressions in mycorrhizal cucumber plants, or phosphorus deficiency in non-mycorrhizal plants, the lack of benefits derived from an effective mycorrhizal symbiosis in the early development of the plant might have reduced the rhizosphere populations of the added *Pseudomonas* spp. This might also explain why the mycorrhizosphere effect was not as consistent in older cucumber plants. These older plants probably had adequate photosynthetic capacity to support both the mycorrhizal symbiont and populations of fluorescent pseudomonads, and older plants might have derived some benefit from the external hyphae of the mycorrhizal fungus.

This reduction in bacterial populations was not evident in roots colonized by *G. etunicatum*, which was a slower root colonizer, and formed fewer vesicles and other fungal biomass. This indicates that the response of rhizosphere populations to VA mycorrhizal fungi cannot be generalized, but is probably dependent on the host response to the fungal species.

Differences in rhizosphere microbial populations between mycorrhizal and non-mycorrhizal plants are documented (Bagyaraj & Menge, 1978; Newman, Heap & Lawley, 1981; Ames, Reid & Ingham, 1984, Ames, Mihara & Bethlenfalvay 1987; Secilia & Bagyaraj, 1987). Most of these studies dealt with general microbial groups, and used general non-specific media that probably favoured organisms that could grow in high-nutrient conditions. Meyer & Linderman (1986*b*) analysed more specific taxonomic and functional groups of bacteria associated with VA mycorrhizal and non-mycorrhizal plants. They found higher populations of chitinase-producers in the rhizosphere of non-mycorrhizal sweet corn, and reported that *Streptomyces* spp. were more abundant in the rhizosphere of non-mycorrhizal clover. They also found lower populations of fluorescent pseudomonads in the rhizosphere of mycorrhizal subterranean clover, when compared to non-mycorrhizal plants, similar to our results. In another set of experiments, a rifampin-resistant strain of *P. putida* (R-20) was added to a natural soil containing an indigenous VA mycorrhizal fungus, which was planted with subterranean clover (Meyer & Linderman, 1986*a*). After 12 weeks, no differences in rhizosphere or rhizoplane populations were detected between mycorrhizal and non-mycorrhizal plants. We observed similar results with this strain, although a different host, fungus, and inoculum density were used. Mycorrhizal colonization was much lower in their experiments, reaching only 7% after 6 weeks. To our knowledge, the results reported here are the first demonstration that VA mycorrhizal fungi can affect the population of a specific bacterial biocontrol strain in the rhizosphere.

The negative to neutral effect of VA mycorrhizal fungi on these biocontrol agents contrasts with other work which demonstrated that VA mycorrhizal fungi stimulated populations and activity of biocontrol agents (Secilia & Bagayaraj, 1987; Meyer & Linderman, 1986*b*).

Bacterial strains 3871 and 2–79 both delayed germination of *G. etunicatum* spores, although the effect disappeared after day 7. Both strains produce potent antifungal compounds. Strain 3871, derived from PF5, produces pyoluteorin and pyrrolinitrin, which are effective against *Pythium ultimum* and *Rhizoctonia solani*, respectively (Howell & Stipanovic, 1979, 1980). Strain 2–79 produces a phenazine compound, which is active against *Gaeumannomyces graminis* var. *tritici* (Gurusiddaiah *et al.*, 1986). To our knowledge, no studies have examined the effects of these antibiotics on VA mycorrhizal fungi. Krishna *et al.* (1982) observed that *Streptomyces cinnamomeous*, an actinomycete that inhibited the growth of plant pathogenic fungi, reduced the sporulation and infection of *Glomus fasciculatum* on finger millet. However, in our research, no effect was seen on the subsequent colonization of cucumber roots by *G. etunicatum* in the presence of high populations of these bacteria (Fig. 6). This neutralistic relationship contrasts with the results of Meyer & Linderman (1986*a*), who reported a stimulation in VA mycorrhizal infection and shoot dry weight of mycorrhizal subterranean clover at 6 weeks. However, this effect disappeared at 12 weeks. They also reported a stimulation in iron uptake and rhizobia nodulation in mycorrhizal plants treated with R-20.

Our results indicate that fluorescent pseudomonads might be compatible with mycorrhizal fungi, and that agricultural use of fluorescent pseudomonads as seed treatments would not adversely affect this symbiosis. However, this premise must

yet be tested with other VA mycorrhizal fungi, soil types, and environmental conditions. Our research also suggests the possibility of an inoculum with a combination of biological control agents and mycorrhizal fungi. The cost of producing a combined inoculum would not be much more than the costs of a single inoculum. Although inoculum of VA mycorrhizal fungi is presently available commercially, the cost per plant is not economically feasible for many crops. In the future, however, increased demand might lower the costs. In addition, this combined microbial inoculum would have multiple benefits: it would maximize plant growth through biological control of pathogens; stimulate plant growth via microbial products, and enhance nutrient uptake by plants.

REFERENCES

AMES, R. N., MIHARA, K. L. & BETHLENFALVAY, G. J. (1987). The establishment of microorganisms in vesicular–arbuscular mycorrhizal and control treatments. *Biology and Fertility of Soils* **3**, 217–223.

AMES, R. N., REID, C. P. P. & INGHAM, E. R. (1984). Rhizosphere bacterial populations responses to root colonization by a vesicular–arbuscular mycorrhizal fungus. *New Phytologist* **96**, 555–563.

BAGYARAJ, D. J. & MENGE, J. A. (1978). Interactions between a VA mycorrhiza and *Azotobacter* and their effects on rhizosphere microflora and plant growth. *New Phytologist* **80**, 567–573.

BARBER, D. A. & LYNCH, J. M. (1977). Microbial growth in the rhizosphere. *Soil Biology and Biochemistry* **9**, 305–308.

BETHLENFALVAY, G. J., BROWN, M. S. & PACOVSKY, R. S. (1982). Parasitic and mutualistic associations between a mycorrhizal fungus and soybean: development of the host plant. *Phytopathology* **72**, 889–893.

BUWALDA, J. G. & GOH, K. M. (1982). Host fungus competition for carbon as a cause of growth depressions in vesicular–arbuscular mycorrhizal ryegrass. *Soil Biology and Biochemistry* **14**, 103–106.

CARON, M., FORTIN, J. A. & RICHARD, C. (1986). Effect of phosphorus concentration and *Glomus intraradices* on *Fusarium* crown and root rot of tomatoes. *Phytopathology* **76**, 942–946.

COX, G., SANDERS, F. E., TINKER, P. B. & WILD, J. A. (1975). Ultrastructural evidence relating to host-endophyte transfer in a vesicular-arbuscular mycorrhiza. In: *Endomycorrhizas* (Ed. by F. E. Sanders, B. Mosse & P. B. Tinker), pp. 297–313. Academic Press, London.

DUPLER, M. & BAKER, R. (1984). Survival of *Pseudomonas putida*, a biological control agent, in soil. *Phytopathology* **74**, 195–200.

GURUSIDDAIAH, S., WELLER, D. M., SARKAR, A. & COOK, R. J. (1986). Characterization of an antibiotic produced by a strain of *Pseudomonas fluorescens* inhibitory to *Gaeumannomyces graminis* var. *tritici* and *Pythium* spp. *Antimicrobial Agents and Chemotherapy* **29**, 488–495.

HARRIS, D., PACOVSKY, R. S. & PAUL, E. A. (1985). Carbon economy of soybean, *Rhizobium*, *Glomus* associations. *New Phytologist* **101**, 427–440.

HEWITT, E. J. (1966). Sand and water culture methods used in the study of plant nutrition. *Technical Communication no. 22*, 2nd edn. Commonwealth Agricultural Bureau, London.

HOWELL, C. R. & STIPANOVIC, R. D. (1979). Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. *Phytopathology* **69**, 480–482.

HOWELL, C. R. & STIPANOVIC, R. D. (1980). Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. *Phytopathology* **70**, 712–714.

KAWAI, Y. & YAMAMOTO, Y. (1986). Increase in the formation and nitrogen fixation of soybean nodules by vesicular-arbuscular mycorrhiza. *Plant Cell Physiology* **27**, 399–405.

KING, E. O., WARD, M. K. & RANEY, D. E. (1954). Two simple media for the demonstration of pyocyanin and fluorescein. *Journal of Laboratory and Clinical Medicine* **44**, 301–307.

KLOEPPER, J. W., LEONG, J., TEINTZE, M., & SCHROTH, M. N. (1980*a*). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* (*London*) **286**, 885–886.

KLOEPPER, J. W., LEONG, J., TEINTZE, M. & SCHROTH, M. N. (1980*b*). *Pseudomonas* siderophores: A mechanism explaining disease-suppressive soils. *Current Microbiology* **4**, 317–320.

KRISHNA, K. R., BALAKRISHNA, A. N. & BAGYARAJ, D. J. (1982). Interactions between a vesicular-arbuscular mycorrhizal fungus and *Streptomyces cinnamomeous* and their effects on finger millet. *New Phytologist* **92**, 401–405.

LINDERMAN, R. G. (1988). Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. *Phytopathology* **78**, 366–371.

MEYER, J. R. & LINDERMAN, R. G. (1986*a*). Response of subterranean clover to dual inoculation with vesicular–arbuscular mycorrhizal fungi and a plant growth-promoting bacterium, *Pseudomonas putida*. *Soil Biology and Biochemistry* **18**, 185–190.

MEYER, J. R. & LINDERMAN, R. G. (1986*b*). Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. *Soil Biology and Biochemistry* **18**, 191–196.

NELSEN, C. E. (1987). The water relations of vesicular–arbuscular mycorrhizal systems. In: *Ecophysiology of VA Mycorrhizal Plants* (Ed. by G. R. Safir), pp. 71–91. CRC Press, Boca Raton, Florida.

NEWMAN, E. I., HEAP, A. J. & LAWLEY, R. A. (1981). Abundance of mycorrhizas and root-surface micro-organisms of *Plantago lanceolata* in relation to soil and vegetation: a multivariate approach. *New Phytologist* **89**, 95–108.

PANG, P. C. & PAUL, E. A. (1980). Effects of vesicular-arbuscular mycorrhiza on ^{14}C and ^{15}N distribution in nodulated fababeans. *Canadian Journal of Soil Science* **60**, 241–250.

PARK, C. S., PAULITZ, T. C. & BAKER, R. (1988). Biocontrol of Fusarium wilt of cucumber resulting from interactions between *Pseudomonas putida* and nonpathogenic isolates of *Fusarium oxysporum*. *Phytopathology* **78**, 190–194.

PAULITZ, T. C. & LINDERMAN, R. G. (1989). Interactions between VA mycorrhizal fungi and fluorescent pseudomonads in the rhizosphere. *Phytopathology* **79**, 1567 (abstr.)

PAULITZ, T. C. & LINDERMAN, R. G. (1990). Mycorrhizal interactions with soil organisms. In: *Handbook of Applied Mycology* (Ed. by D. K. Arora). Marcel-Dekker, New York (in the press).

PHILLIPS, J. M. & HAYMAN, D. S. (1970). Improved procedure for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**, 158–161.

SCHER, F. M. & BAKER, R. (1982). Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to Fusarium wilt pathogens. *Phytopathology* **72**, 1567–1573.

SECILIA, J. & BAGYARAJ, D. J. (1987). Bacteria and actinomycetes associated with pot cultures of vesicular–arbuscular mycorrhizas. *Canadian Journal of Microbiology* **33**, 1069–1073.

STEEL, R. G. D. & TORRIE, J. J. (1980). *Principles and Procedures of Statistics*, 2nd Ed. McGraw-Hill Book Co., New York.

SUBBA RAO, N. S., TILAK, K. V. B. R., & SINGH C. S. (1986). Dual inoculation with *Rhizobium* sp. and *Glomus fasciculatum* enhances nodulation, yield and nitrogen fixation in chickpea (*Cicer arietinum* Linn.) *Plant and Soil* **95**, 351–359.

SUSLOW, T. V. & SCHROTH, M. N. (1982). Rhizobacteria of sugarbeets: effects of seed application and root colonization on yield. *Phytopathology* **72**, 199–206.

TINKER, P. B. (1984). The role of microorganisms in mediating and facilitating the uptake of plant nutrients from soil. *Plant and Soil* **76**, 77–91.

WELLER, D. M. & COOK, R. J. (1983). Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. *Phytopathology* **73**, 463–469.