Plant and Soil **202:** 79–87, 1998.

Bacterial associations with the mycorrhizosphere and hyphosphere of the arbuscular mycorrhizal fungus *Glomus mosseae*

G. Andrade[1], R.G. Linderman[2] and G.J. Bethlenfalvay[2,3]
[1]*Universidade Estadual de Londrina, Depto. de Microbiologia, Londrina, PR 86051-970, Brazil, and* [2]*U.S. Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Laboratory, 3420 NW Orchard Avenue, Corvallis, OR 97330, USA.* [3]*Corresponding author**

Received 30 June 1997. Accepted in revised form 10 April 1998

Key words: Alcaligenes, *Arthrobacter*, *Glomus mosseae*, hyphosphere, mycorrhiza, mycorrhizosphere, *Pseudomonas*, rhizobacteria, rhizosphere, *Sorghum*

Abstract

Roots and mycorrhizal fungi may not associate with soil bacteria randomly, but rather in a hierarchical structure of mutual preferences. Elucidation of such structures would facilitate the management of the soil biota to enhance the stability of the plant-soil system. We conducted an experiment utilizing two isolates of soil bacteria to determine their persistence in distinct mycorrhizal regions of the root zone, and their effects on general rhizosphere populations of fluorescent pseudomonads (FP). Split-root sorghum (*Sorghum bicolor* L.) plants were grown in four-compartment containers, constructed so that the soils in individual compartments held either (1) roots colonized by the arbuscular-mycorrhizal (AM) fungus *Glomus mosseae* (M), (2) nonAM roots only (R), (3) hyphae of *G. mosseae* (H), or (4) no mycorrhizal structures (S). The soils were inoculated (10^7 cells g^{-1} dry soil) with antibiotic-resistant (rifampicin, rif; streptomycin, sm) strains of the soil bacteria, *Alcaligenes eutrophus* ($rif^r 50$) or *Arthrobacter globiformis* ($sm^r 250$), or were left uninoculated as control. *A. eutrophus* had been isolated from a specific source (hyphosphere soil of *G. mosseae*), and *A. globiformis* from mycorrhizosphere soils of two AM fungi. After 10 wk of growth, the presence of *A. eutrophus* was barely detectable (<10 cfu g^{-1} dry soil) in nonAM (R and S) soils, but persisted well (10^4 cfu g^{-1} dry soil) in AM (H and M) soils. Numbers of *A. globiformis* were more evenly distributed between all soils, but were highest in the presence of AM roots (M soil). There were varied bacterial effects on root and AM-hyphal development: *A. eutrophus* decreased hyphal length in H soil, while *A. globiformis* stimulated root length in M soil. The two bacterial inoculants did not affect numbers of FP in H, R, and M soils, but the AM status of the soils did: the numbers of FP increased in the order M>R>H>S. There was a positive correlation of FP numbers with both bacterial inoculants in M and H soils. Numbers of FP changed with root or hyphal lengths, an effect that was related to changes in the numbers of the inoculated bacteria. The results indicate that the hyphosphere-specific *A. eutrophus* depended on the presence of *G. mosseae*, but that the nonspecific *A. globiformis* did not. The mycorrhizal status of soils may selectively influence persistence of bacterial inoculants as well as affecting the numbers of other native bacteria.

Introduction

The rhizosphere (Hiltner, 1904) had been defined for almost a century as the narrow zone of soil subject to the influence of living roots, characterized by intense bacterial activity as a result of a leakage or exudation of substances from the root (Curl and Truelove, 1986). Subsequently, the rhizosphere concept was expanded to include the root's ever-present mycorrhizal fungal associates (Rawlings, 1958). In the mycorrhizosphere (Oswald and Ferchau, 1968), two different zones are discernible: that under the joint influence of the root and fungal components of the mycorrhiza, and that affected by the mycelium of the mycorrhizal fungus

* FAX No.: 1 541 750 8764. E-mail: bethleng@ucs.orst.edu

only. This second zone, the hyphosphere (Marschner, 1995), is part of the over-all mycorrhizosphere but may support biotic activities distinct from those in soils under joint root and fungal influence (Linderman, 1988). Since microbial activity may differ in mycorrhizosphere, hyphosphere, rhizosphere and bulk soils (Andrade et al., 1997), we will here use the terms 'rhizosphere' to refer to soil surrounding nonmycorrhizal roots only, and 'bulk soil' to refer to soil free of both the plant and fungal components of the mycorrhiza (Andrade et al., 1997).

Reports of interactions between microorganisms and arbuscular mycorrhizal (AM) fungi include both negative and positive effects of the fungi on bacterial activity: *Glomus fasciculatum* in association with *Zea mays* or *Trifolium subterraneum* reduced the viable counts of fluorescent pseudomonads (FP) but increased total bacterial numbers compared to nonAM plants (Meyer and Linderman, 1986), while the total viable counts of bacteria in the rhizoplane of guinea grass was reduced by *Acaulospora laevis* but increased by *G. fasciculatum* (Secilia and Bagyaraj, 1987). Differences between bacterial communities in rhizosphere, hyphosphere, and mycorrhizosphere soils are also expected to exist due to quantitative and qualitative differences in nutrients derived from the plant, the fungus, or from both, but so far the spatial differentiation of bacteria in the root zone has been only superficially explored (Andrade, 1997; Olsson et al., 1996).

Andrade et al. (1997) found qualitative differences in the composition of the microflora in mycorrhizosphere and hyphosphere soils of different AM fungal isolates, suggesting preferential associations between some bacteria and the conditions provided by their individual fungal hosts. Bacterial colonization of the mycorrhizosphere and hyphosphere may thus differ with the AM fungi that colonize a given plant's root zone. Since microbial activity in soil is stimulated by root exudates (Bowen and Rovira, 1991), root colonization by AM fungi may alter bacterial growth by changing exudation patterns (Azaizeh et al., 1995). The fungi themselves may promote bacterial growth in the soil directly, by exuding nutrients transferred from the roots to soil microsites not accessible to roots (Hobbie, 1992; Söderström, 1992).

Deleterious effects of AM root and soil colonization on soil bacteria have also been observed, suggesting C competition (Marschner and Crowley, 1996; Paulitz and Linderman, 1989). But other interpretations of AM-fungal inhibition of distinct bacterial populations, functional groups, or taxa also exist and include: (1) the influence of the fungi on the patterns and availability of plant-derived C-sources and their utilization by microbial communities (Garland, 1996; Washkies et al., 1994), (2) the enhancement of competing bacterial populations (Ames et al., 1984), (3) the creation of unfavorable physico-chemical soil conditions, and (4) the stimulation of predator populations. An understanding of such multiple-factor interactions and their reduction to the most plausible cause-effect relations would be facilitated if hierarchical specificity or preference structures could be elucidated between associated organisms. Such preferences have been shown to exist between certain plants and AM fungi (ecological specificity, McGonigle and Fitter, 1990) and are suggested by the occurrence of distinct bacterial communities associated with mycorrhizosphere and hyphosphere soils of different AM fungi (Andrade et al., 1997).

The purpose of the present study was to assess: (1) the preference of two bacterial strains isolated from the hyphosphere or mycorrhizosphere of the AM fungus *G. mosseae* for the soil zone from which they were isolated, (2) the effects of the massive inoculation of these organisms on mycorrhiza development, and (3) the proliferation of a third soil bacterial type, FP, that is a general rhizosphere inhabitant.

Material and methods

Experimental unit and design

Plants were grown in containers consisting of four compartments described elsewhere (Andrade et al., 1998). Roots were split over a central solid barrier and trained to grow into the soils of the two (inside) compartments on either side of the solid barrier. The soil in one of the inside compartments was inoculated with an arbuscular-mycorrhizal (AM) fungus; the other was not (nonAM). The soils of each of these two inside compartments was contiguous with the soil of an outside compartment but were separated from the outside compartment by a screen (43 μm). The screens prevented root penetration into the two outside compartments but permitted the growth of hyphae and an exchange of the soil microflora and of the soil solution. The four compartments thus contained either bulk soil (S, no roots, no AM hyphae, outside compartment) or rhizosphere soil (R, nonAM roots only, inside compartment) on the nonAM side, and hyphosphere

soil (H, AM hyphae only, outside compartment) or mycorrhizosphere soil (M, AM roots and AM hyphae, inside compartment) on the AM side of the central solid barrier.

There were nine such experimental units, each containing S, R, M and H soils. The soils (≈1 kg per compartment) were spread out in a thin (1 cm) layer before potting and sprayed with suspensions (10^7 cells g^{-1} soil) of the Gram-negative bacterium *Alcaligenes eutrophus* (Ae) or the Gram-positive actinobacterium *Arthrobacter globiformis* (Ag) or with the sterile medium (Ringer's solution) as control (CON). Each suspension was used to treat all of the four soils of three of the nine experimental units. This procedure resulted in 12 treatments in a 3×4 factorial design, with bacterial inoculation (Ae, Ag and CON) and mycorrhizal status (S, R, M and H) as factors. Upon removal from the containers, the soil clumps were cut in two, and each half was sampled separately. The data from these subsamples were averaged and the resulting three replications were used to evaluate treatments by analysis of variance, regression analysis, and orthogonal contrasts. We presented actual probability values (p) instead of arbitrary probability levels ($p \leq 0.05$ or $p \leq 0.01$) where applicable, to permit the reader to interpret significance (Nelson, 1989).

Growth conditions

Each compartment was filled with 1.5 L (1 kg) of a steam-pasteurized (75 °C, 3 h) mix of sand and sandy-loam soil (v:v, 1:1) described elsewhere (Andrade et al., 1998). Spores of the AM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd and Trappe (INVAM[1] isolate # CA 110) were obtained from soil cultures with sorghum (*Sorghum bicolor* L.), surface sterilized, and tested for germination rates. The soils of the M compartments were inoculated with 150 of these spores. Surface-sterilized (ethanol, 70%, v:v, 2 min), pre-germinated sorghum seeds were used, and the roots of the seedlings were split to grow into R- and M-compartment soils (Andrade et al., 1998).

Plants were grown in a greenhouse at Corvallis, OR, and harvested after 10 wk of growth. The soils of the inside (R and M) compartments were watered daily with tap water and those of the outside compartments (S and H) once a week to keep them at similar moisture contents (field capacity). Sunlight was supplemented by 1000 W phosphor-coated metal halide lamps, providing 16 h of photosynthetically active radiation (450 μmol m^{-2} sec^{-1}) at soil surface level. Temperatures were kept within 18 and 21 °C by automatic controls.

Marking and inoculation with mycorrhizosphere and hyphosphere bacteria

The strains of bacteria used as inoculants were isolated from our experimental soil. Isolates of *A. eutrophus* were obtained from the hyphosphere and those of *A. globiformis* from the mycorrhizosphere of sorghum roots colonized by *G. mosseae* (Andrade et al., 1997). The *A. eutrophus* strain occurred only in the hyphosphere of *G. mosseae*, while the *A. globiformis* strain originally colonized rhizosphere and hyphosphere soils of more than one AM fungus. In addition to the hyphosphere of *G. mosseae*, it was also found in the bulk soil of nonAM plants, and in the mycorrhizosphere of another AM fungus, *Glomus intraradices* (Schenck and Smith) Koske. Stable antibiotic-resistant mutants of *A. eutrophus* and *A. globiformis* were obtained spontaneously by exposure to rifampicin (rif) and streptomycin (sm), respectively.

The mutants were obtained as follows. Concentrated (10^{10} cfu mL^{-1}) cell suspensions of *A. eutrophus* and *A. globiformis* were exposed to concentration gradients of rif (25–150 μg mL^{-1}) or sm (50–250 μg mL^{-1}) on Tryptic-Soy Agar (TSA) medium and incubated at 25 °C for 10 d. Bacteria surviving the highest concentrations of the bactericides were subjected five times to single-colony isolation on antibiotic-supplemented TSA to test for stability before soil inoculation. The inocula were prepared in TSA broth (plus rif 50 or sm 250). The cells were washed three times with one-quarter strength Ringer's solution (centrifugation, 10,000 *g*, 20 min), resuspended in Ringer's solution and sprayed on thin layers of soil to obtain a final soil concentration of approximately 10^7 cfu g $soil^{-1}$. The inoculated soils were mixed before potting.

Isolation of A. eutrophus, A. globiformis *and fluorescent pseudomonads (FP)*

Two 1-g samples of homogenized soil were taken from each of the 12 treatments to estimate the populations of *A. eutrophus* rif^r 50, *A. globiformis* sm^r 250 and native FP (Zuberer, 1994). Samples from R and M compartments also contained root fragments. The samples were suspended in 9 mL of one-quarter

[1] International Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi, Division of Plant and Soil Sciences, University of West Virginia, Morgantown, WV 26526-6057, USA.

Table 1. Statistical evaluation of mycorrhiza effects on populations of the soil bacteria *A. eutrophus* and *A. globiformis*. All soils (bulk soil, S; rhizosphere soil, R; mycorrhizosphere soil, M; and hyphosphere soil, H) were inoculated with one of the two bacteria (10^7 cfu g^{-1} dry soil)

Bacteria	ANOVA	Orthogonal contrasts					
		S vs. R	S vs. M	S vs. H	R vs. M	R vs. H	M vs. H
A. eutrophus	<0.001	0.858	<0.001	<0.001	<0.001	<0.001	0.869
A. globiformis	0.020	0.437	0.159	0.713	0.045	0.673	0.089

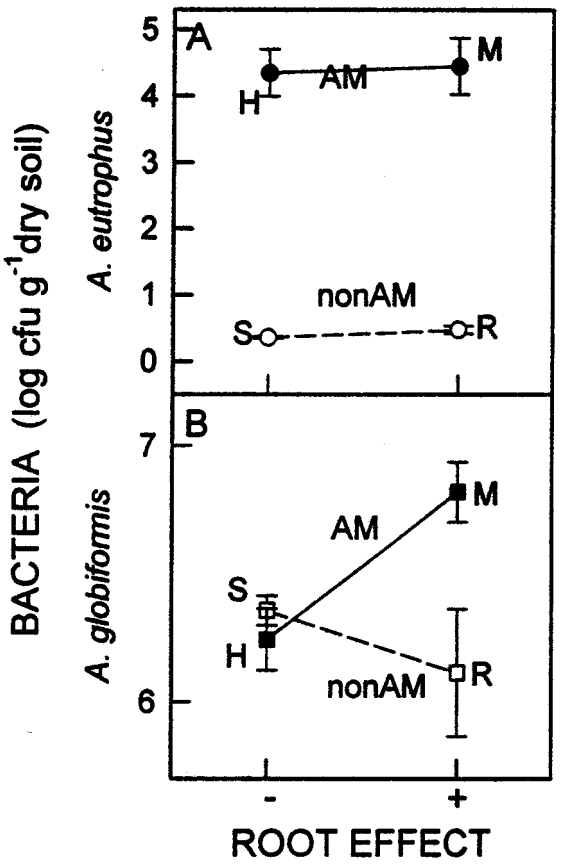

Figure 1. Graphical representation of two-factor interactions for bacterial numbers of *Alcaligenes eutrophus* and *Arthrobacter globiformis* in soils of different mycorrhizal status. A. Points denoting bacteria numbers in hyphosphere (H) and mycorrhizosphere (M) soils, and in bulk soil (S) and rhizosphere soil (R) are connected to illustrate that the Mycorrhiza Factor is the same at each level of the Root Factor. B. Points denoting bacteria numbers (as above) are connected to illustrate the failure of the Mycorrhiza Factor to be the same at each level of the Root Factor.

strength Ringer's solution (plus 0.05% agar) and maintained at 5 °C until the serial dilutions were made. Aliquots (100 μL) of ten-fold dilutions were spread on duplicate plates of TSA medium plus rifampicin 50 μg mL^{-1} or streptomycin 250 μg mL^{-1} for counting the marked strains (Kirchner et al., 1993) and on P1 medium (Katoh and Itoh, 1983) for the FP. Plates were incubated at 25 °C and cfu were counted after 5 d.

Mycorrhiza assay

Roots were washed and cut into 1-cm segments. Subsamples (1 g) were cleared in KOH solution (5% KOH, w:v, 30 min, 90 °C), and stained with trypan blue (0.05%) in lacto-glycerol (water:glycerol:lactic acid, 1:1:1, 10 min, 90 °C). Total and AM-colonized root length was estimated by the grid-line intersect method (Giovanetti and Mosse, 1980). Two samples from each treatment soil were used for a determination of fungal hyphal length by the grid-line intersect method (Sylvia, 1992), as modified by Andrade et al. (1998). Hyphal length was determined in the nonAM treatments also and these hyphae were considered to be residual dead AM and nonAM hyphae. This value was subtracted from the hyphal length of the AM treatments.

Results

A. eutrophus *rif^r 50 and* A. globiformis *sm^r 250 populations*

The mycorrhizal status of the soils affected the marked populations of *A. eutrophus* and of *A. globiformis* differently (Figure 1, Table 1). *A. eutrophus* showed a marked preference for soils containing AM hyphae (M and H), and its numbers therein exceeded those found in the nonAM (S and R) soils by four orders of magnitude. Populations of *A. globiformis* were more evenly distributed, but were higher in the M than in the other soils. The numbers of both *A. eutrophus* and *A. globiformis* declined in all soil treatments from the original

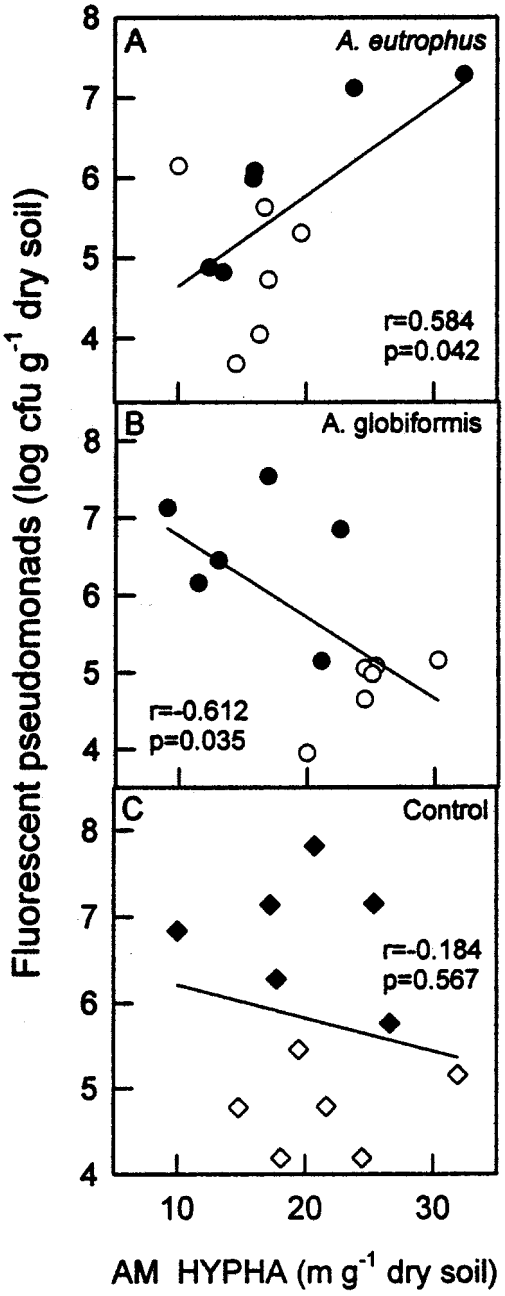

Figure 2. Relationship of fluorescent pseudomonads (FP) with hyphal length of the arbuscular-mycorrhizal (AM) fungus *Glomus mosseae* in soils inoculated with antibiotic-resistance marked isolates of *Alcaligenes eutrophus* or *Arthrobacter globiformis*. A data point (two samples from each of the three replications) represents one observation in mycorrhizosphere (closed symbols) or hyphosphere (open symbols) soils.

value (10^7 cfu g^{-1} dry soil). The near elimination of *A. eutrophus* from nonAM soils showed a preference of this organism for AM hyphae, from which it was isolated, and in whose presence it persisted throughout the experiment in numbers ten thousand-fold higher than in their absence. On the other hand, *A. globiformis*, a nonspecific colonist of the bulk soil (Andrade et al., 1997), persisted well in all soils, although with a preference for the M soil.

The difference in the responses of these bacteria to soils of different AM status are best shown by the graphical representation of the interactions provided by ANOVA (Figure 1). When evaluated as a 2 × 2 factorial with AM fungus (presence [H, M] or absence [S, R]) and host root (presence [R, M] or absence [S, H]) as factors, the effect of the AM factor on *A. eutrophus* was the same at each level of the root factor. The factors were therefore independent of one another, the interaction was not significant (p = 0.992), and it was the AM (and not the root) status of the soil that affected the proliferation of this organism (Figure 1A). For *A. globiformis*, on the other hand, the AM effect was different at each level of the root factor (Figure 1B). Thus, the factors were not independent of one another, the responses were different in direction and magnitude, and the interaction was significant (p = 0.087). In this case, bacterial numbers were affected by both the AM and the root status of the soils.

AM responses to bacteria

Bacterial inoculation had significant effects on some, but not all, of the root and AM traits (Table 2). AM hyphal length in hyphosphere soil was lower in the Ae treatment than with the other two treatments, while in mycorrhizosphere soils bacterial treatments had no significant effects on hyphal development. The presence of Ag stimulated hyphal development in the hyphosphere, and inhibited it in the mycorrhizosphere. In rhizosphere soil, root length in the Ag treatment was lower (p = 0.094) than that of the control treatment. In the mycorrhizosphere, *A. eutrophus* enhanced root growth (p = 0.086) compared to the control. Specific fine root length was most developed in the mycorrhizosphere and significantly enhanced both by *A. eutrophus* (p = 0.034) and by *A. globiformis* (p = 0.007).

Interactions with fluorescent pseudomonads (FP)

Populations of FP were significantly influenced by the AM and root status of the soils (Table 3), which stimulated FP numbers in the order: M>R>H>S. The presence of the AM fungus caused FP numbers to increase by at least an order of magnitude (compare M

Table 2. Root and arbuscular-mycorrhizal (AM) responses to bacterial inoculation. Soils (hyphosphere soil, H; rhizosphere soil, R; and mycorrhizosphere soil, M) were inoculated with the soil bacteria *Alcaligenes eutrophus* (Ae) or *Arthrobacter globiformis* (Ag) at a rate of 10^7 cfu g^{-1} dry soil, or were left uninoculated (control). Tertiary roots were used to determine specific fine root length (SFRL). ANOVA refers to the Bacterium×AM (3×2) factorials

Treatment	AM hypha (m/g dry soil)		Root length (m/kg dry soil)		SFRL (m/kg dry soil)	
	H-soil	M-soil	R-soil	M-soil	R-soil	M-soil
Control	21.8	19.6	72.3	82.9	46.6	48.2
A. eutrophus	15.7	19.0	54.3	123.2	41.5	62.9
A. globiformis	25.0	15.7	42.3	86.1	40.5	94.9
ANOVA (*p*-values)						
Bacterium	0.391		0.261		0.132	
AM	0.223		0.003		0.007	
B×AM	0.090		0.136		0.053	
Orthogonal contrasts (*p*-values)						
C vs. Ae	0.104	0.824	0.427	0.086	0.749	0.258
C vs Ag	0.343	0.261	0.094	0.520	0.706	0.007
Ae vs. Ag	0.026	0.342	0.660	0.255	0.953	0.034

Table 3. Effects of bacterial inoculation and AM status on native populations of fluorescent pseudomonads (FP). Soils (bulk soil, S; rhizosphere soil, R; mycorrhizosphere soil, M; and hyphosphere soil, H) were inoculated with *Alcaligenes eutrophus* (Ae) or *Arthrobacter globiformis* (Ag) at a rate of 10^7 cfu g^{-1} dry soil, or were left uninoculated (control)

Treatment	AM status of soils FP (log cfu g^{-1} dry soil)				ANOVA
	S	H	R	M	
Control	0.38	4.76	5.83	6.83	<0.001
Ae	0.38	4.81	5.64	6.03	<0.001
Ag	3.52	4.92	5.75	6.62	<0.001
ANOVA	<0.001	0.922	0.967	0.312	

vs. R soils, and also H vs. S soils of the Ag treatment). Bacterial inoculation affected FP only in the root-free bulk soil, where it occurred in only very small numbers in the control and Ae treatments, but increased a thousand-fold in the presence of *A. globiformis*.

There was a significant positive correlation between FP and AM hyphal length in the Ae treatment, a negative one in the Ag treatment, while the correlation was not significant in the control (Figure 2). Since the presence of the bacterial inoculants affected hyphal development (Table 2), but not FP numbers (Table 3), the hyphal effect on FP numbers may be interpreted as an indirect effect of the two bacterial inoculants.

In Ae soils, FP numbers increased from very low levels in the bulk soil to the higher levels in the H, R and M soils (Table 3). Since this effect was paralleled in Ae treatments by an increase in hyphal length from zero in the bulk soil (not shown) to the highest level in the M soil (Table 2), the increase in FP numbers (M>H>S) could have been influenced by the proximity of the AM roots or by the more developed AM mycelium. At the same time, protagonism between *A. eutrophus* and FP was also shown by the direct, positive correlation between the two organisms (Figure 3A).

Fluorescent pseudomonads were also positively correlated with *A. globiformis* (Figure 3B), but their relationship with AM hyphal development was negative (Figure 2B), resembling their behavior in the uninoculated control soils (Figure 2C). This comparison indicates that regardless of the presence of *A. globiformis*, it was root, rather than hyphal development, that stimulated FP. In the light of these data, the correlations between FP and hyphal length appear to be due to the effects of *A. eutrophus* and *A. globiformis* on hyphal development.

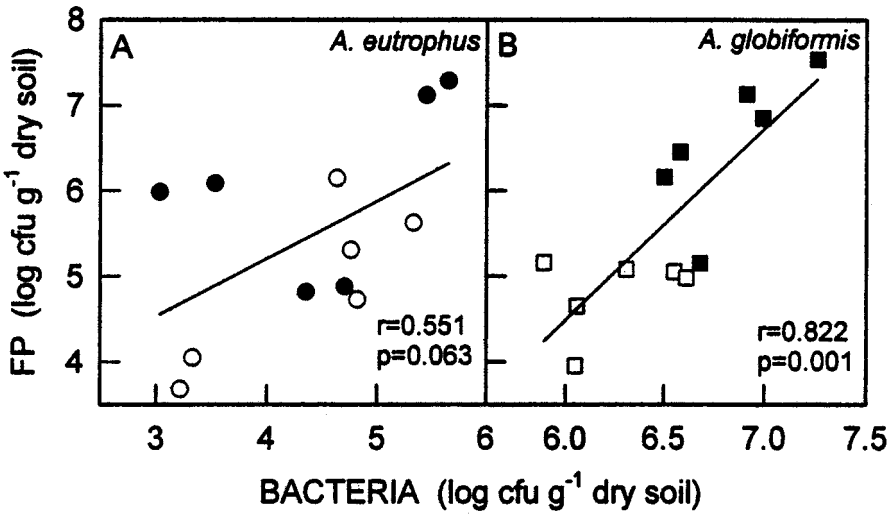

Figure 3. Relationship of fluorescent pseudomonads (FP) with antibiotic-resistance marked isolates of *Alcaligenes eutrophus* and *Arthrobacter globiformis* in mycorrhizosphere (●) or hyphosphere (○) soils.

Discussion

The difference in the proliferation of the soil bacteria *A. eutrophus* and *A. globiformis* in AM or nonAM soils was the salient finding of our experiment. The 'mycorrhizobacterium' *A. eutrophus*, isolated from the hyphosphere of the AM fungus *G. mosseae*, established itself in both hyphosphere and mycorrhizosphere soils in numbers ten thousand times greater than in soils not containing the fungus. Its success in H and M soils did, therefore, not depend on the presence of the host root, but on AM hyphae (Table 1). This effect also suggests that this bacterial isolate has a preference for *G. mosseae* as its AM-fungal host. This preference may not be mutually advantageous, however, as AM hyphal length declined in the presence of the bacterium.

Preferences (ecological specificities) in the mycorrhizosphere have been shown to exist between the host plant and its AM-fungal associates (McGonigle and Fitter, 1990), and they may thus extend to the AM fungus-soil bacterium association also. Such bacterium-AM fungus preferences may not be generalized, however, as shown by the behavior of the nonspecific isolate, *A. globiformis*. Although it produced the greatest cell numbers in mycorrhizosphere soil, it persisted equally well in both AM and nonAM soils, probably due to the improvement in growth-substrate availability by the fungus-root association (Wright and Upadhaya, 1996).

The relationships between the mycorrhiza and a bacterial associate may affect other members of the mycorrhizosphere community. The potential of soil bacteria to control pathogens is being exploited in agriculture (Pankhurst et al., 1994), but the success rate has been inconsistent. There is a need for more data on basic soil-community dynamics (Garland, 1996). Such an entry into the database was provided by our observation that numbers of FP soil bacteria as a function of AM hyphal length differed in the presence of *A. eutrophus* or *A. globiformis*. Different AM fungi have been shown by others to have different effects on populations of FP, a phenomenon that has been attributed to competition for growth substrates, which can lead to a starvation of the bacterium in the rhizosphere (Marschner and Crowley, 1996). It was unexpected, however, to find FP numbers correlated both positively and negatively with hyphal development of the same AM fungus (Figure 2), apparently depending on the presence or absence of *A. eutrophus* or *A. globiformis*. The positive correlations of FP with both *A. eutrophus* and *A. globiformis* (Figure 3), and the lack of a significant relationship between hyphal length and FP in control soil (Figure 2C) indicated that FP numbers were independent of the hyphae. The large increase of FP numbers in S soil treated with *A. globiformis* (Table 3) is obscure, as a similar enhancement was not observed in the other soils.

In a multi-factor system, such as the soil, inferences drawn from insufficient data have little predic-

tive value, because effects observed may be due to, or at least influenced by, yet another little-known, or unknown factor. For example, in considering the composition of FP populations associated with roots, one may conclude that soil type is a dominant factor if AM fungi are not one of the factors evaluated (Latour et al., 1996). When evaluating the effects of introduced soil bacteria on plants, alterations in rooting patterns and AM colonization are noted (Table 2, Ames et al., 1983; Andrade et al., 1995; Germida and Walley, 1996) that may be plant-growth promoting in one sense, but may also alter the optimal functioning of established associations. Such associations may be as manifold as the organisms of which they are composed: plant–fungus, plant–bacterium, fungus–bacterium, and plant–fungus–bacterium associations ranging from the highly specific to the mildly preferential. Upon disturbing the balance of this large assemblage of associations, significant, although transient, perturbations occur (De Leij et al., 1995; Gilbert et al., 1996) that may interrupt natural interactions between soil populations and may prevent a consistent, full exploitation of the benefits derived from inoculation with plant–growth–promoting rhizobacteria or bacterial biocontrol agents (Ryder et al., 1994).

An establishment of a hierarchical system of specificities and preferences that relate associated soil organisms to one another may become necessary as varying levels of interdependence between the members of specific associations become known. Understanding the mechanisms through which soil organisms interact is crucial for the management of sustainable agricultural systems (Hamel, 1996).

References

Ames R N, Reid C P P and Ingham E R 1984 Rhizosphere bacterial population responses to root colonization by a vesicular-arbuscular mycorrhizal fungus. New Phytol. 96, 555–563.

Andrade G, Azcon R and Bethlenfalvay G J 1995 A rhizobacterium modifies plant and soil responses to the mycorrhizal fungus *Glomus mosseae*. Appl. Soil Ecol. 2, 195–202.

Andrade G, Mihara K L, Linderman R G and Bethlenfalvay G J 1997 Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant Soil 192, 71–79.

Andrade G, Mihara K L, Linderman R G and Bethlenfalvay G J 1998 Soil aggregation status and rhizobacteria in the mycorrhizosphere. Plant Soil 202, 89–96.

Azaizeh H A, Marschner A, Römheld V and Wittenmayer L 1995 Effects of a vesicular-arbuscular mycorrhizal fungus and other soil microorganisms on growth, mineral nutrient acquisition and root exudation of soil-grown maize plants. Mycorrhiza 5, 321–327.

Bowen G D and Rovira A D 1991 The rhizosphere. *In* Plant Roots, the Hidden Half. Eds. Y Waisel A Eshel and U Kafkafi. pp 641–669. Marcel Dekker, New York.

Curl E A and Truelove B 1986 The Rhizosphere. pp 1–8. Springer-Verlag, Berlin.

De Leij F A A M, Sutton E J, Whipps J M, Fenlon J S and Lynch J M 1995 Impact of field release of genetically modified *Pseudomonas fluorescens* on indigenous microbial populations on wheat. Appl. Environ. Microbiol. 61, 3443–3453.

Garland J L 1996 Patterns of potential C source utilization by rhizosphere communities. Soil Biol. Biochem. 28, 223–230.

Germida J J and Walley F L 1996 Plant-growth promoting rhizobacteria alter rooting patterns and arbuscular mycorrhizal fungi colonization of field-grown spring wheat. Biol. Fertil. Soils 23, 113–120.

Gilbert G S, Clayton M K, Handelsman J and Parke J L 1996 Use of cluster and discriminant analyses to compare rhizosphere bacterial communities following biological perturbation. Microb. Ecol. 32, 123–147.

Giovanetti M and Mosse B 1980 An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infections in roots. New Phytol. 84, 489–500.

Hamel C 1996 Prospects and problems pertaining to the management of arbuscular mycorrhizae in agriculture. Agric. Ecosyst. Environ. 60, 197–210.

Hiltner L 1904 Über neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie und unter besonderer Berüksichtigung der Gründüngung und Brache (On recent insights and problems in the area of soil bacteriology under special consideration of the use of green manure and fallowing). Arb. Dtsch. Landwirt. Ges. 98, 59–78.

Hobbie S E 1992 Effects of plant species on nutrient cycling. Trends Ecol. Evol. 7, 336–339.

Katoh K and Itoh K 1983 New selective media for Pseudomonas strains producing fluorescent pigment. Soil Sci. Plant Nutr. 29, 525–532.

Kirchner M J, Wollum II A G and King L D 1993 Soil microbial populations and activities in reduced chemical input agroecosystems. Soil Sci. Soc. Am. J. 57, 1289–1295.

Latour X, Corberand T, Laguerre G, Allard F and Lemanceau P 1996 The composition of fluorescent pseudomonad populations associated with roots is influenced by plant and soil type. Appl. Envir. Microbiol. 62, 2449–2456.

Linderman R G 1988 Mycorrhizal interactions with the rhizosphere microflora: The mycorrhizosphere effect. Phytopathology 78, 366–371.

Marschner H 1995 Mineral Nutrition of Higher Plants. 2nd ed. Academic Press, London.

Marschner P and Crowley D E 1996 Physiological activity of a bioluminescent *Pseudomonas fluorescens* (strain 2-79) in the rhizosphere of mycorrhizal and non-mycorrhizal pepper (*Capsicum annum* L.). Soil Biol. Biochem. 28, 869–876.

McGonigle T P and Fitter A H 1990 Ecological specificity of vesicular-arbuscular mycorrhizal associations. Mycol. Res. 94, 120–122.

Meyer J R and Linderman R G 1986 Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. Soil Biol. Biochem. 18, 191–196.

Nelson L A 1989 A statistical editor's viewpoint of statistical usage in horticultural science publications. HortScience 24, 53–57.

Olsson P A, Baath E, Jakobsen I and Söderström B 1996 Soil bacteria respond to presence of roots but not to mycelium of arbuscular mycorrhizal fungi. Soil Biol. Biochem. 28, 463–470.

Oswald E T and Ferchau H A 1968 Bacterial associations of coniferous mycorrhizae. Plant Soil 28, 187–192.

Pankhurst C E, Double B M, Gupta V V S R and Grace P R (Eds.) 1994 Soil Biota, Management in Sustainable Farming Systems. CSIRO Information Services, East Melbourne, VI, Australia.

Paulitz T C and Linderman R G 1989 Interactions between fluorescent pseudomonads and VA mycorrhizal fungi. New Phytol. 113, 37–45.

Rawlings, G B 1958 Some practical aspects of forest mycotrophy. New Zealand Soc. Soil Sci. Proc. 3, 41–44.

Ryder M H, Stephens P M and Bowen G D (Eds.) 1994 Improving Plant Productivity with Rhizosphere Bacteria. CSIRO Division of Soils, Glen Osmond, SA, Australia.

Secilia J and Bagyaraj D J 1987 Bacteria and actinomycetes associated with pot cultures of vesicular-arbuscular mycorrhizas. Can. J. Microbiol. 33, 1067–1073.

Söderström B 1992 The ecological potential of the ectomycorrhizal mycelium. *In* Mycorrhizas in Ecosystems. Eds. D J Read, D H Lewis, A H Filter and I J Alexander. pp 77–83. CAB International, Wallingford, UK.

Sylvia D M 1992 Quantification of external hyphae of vesicular-arbuscular mycorrhizal fungi. Methods Microbiol. 24, 53–65.

Waschkies C, Schroop A and Marschner H 1994 Relations between grapevine replant disease and root colonization of grapevine (*Vitis* sp.) by fluorescent pseudomonas and endomycorrhizal fungi. Plant Soil 162, 219–227.

Wright S F and Upadhaya A 1996 Extraction of an abundant and unusual protein from soil and comparison with hyphal protein for arbuscular mycorrhizal fungi. Soil Sci. 161, 575–586.

Zuberer D A 1994 Recovery and enumeration of viable bacteria. *In* Methods of Soil Analysis, Part 2. American Society of Agronomy, Madison, WI.

Section editor: M Jones