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Response of spring wheat (*Triticum aestivum*) to interactions between *Pseudomonas* species and *Glomus clarum* NT4

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Abstract The effects of interactions between pseudomonads (*Pseudomonas cepacia* strains R55 and R85, *P. aeruginosa* strain R80, *P. fluorescens* strain R92, and *P. putida* strain R104) and the arbuscular mycorrhizal fungus *Glomus clarum* (Nicol. and Schenck) isolate NT4, on spring wheat (*Triticum aestivum* L. cv. Laura), grown under gnotobiotic and nonsterile conditions, were investigated. Although plant growth responses varied, positive responses to pseudomonad inoculants generally were obtained under gnotobiotic conditions. Shoot dry weight enhancement ranged from 16 to 48%, whereas root enhancement ranged from 82 to 137%. Shoot growth in nonsterile soil, however, was unaffected by pseudomonad inoculants, or reduced by as much as 24%. Shoot growth was unaffected or depressed by *G. clarum* NT4 whereas early root growth was enhanced by 38%. Significant interactions between the pseudomonad inoculants and *G. clarum* NT4 were detected. Typically, dual inoculation influenced the magnitude of response associated with any organism applied alone. The effect of these pseudomonads on *G. clarum* NT4 spore germination was investigated. Germination was inhibited when spores were incubated either on membranes placed directly on bacterial lawns of strains R85 and R104 (i.e., direct assay), or on agarose blocks separated from the bacteria by membranes (i.e., diffusion assay). When the agarose blocks were physically separated from the pseudomonad (i.e., volatile assay), there was no evidence of inhibition, suggesting that a nonvolatile, diffusible substance(s) produced by both strains R85 and R104 may inhibit *G. clarum* NT4 spore germination.

Key words *Glomus clarum* · *Pseudomonas* spp. · Arbuscular mycorrhizae · Rhizobacteria · Spore germination · Gnotobiotic conditions · Inoculation

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Introduction

Considerable interest and research has been directed towards enhancing plant growth by manipulating the microbial status of the rhizosphere via the introduction of plant growth-promoting rhizobacteria (PGPR), many of which have been identified as pseudomonads (Kloepper et al. 1989). Although pseudomonad-induced plant growth-promotion has been attributed to production of plant growth-regulating substances (Lifshitz et al. 1987; Kloepper et al. 1988), siderophore- and antibiotic-mediated biocontrol of deleterious or pathogenic microorganisms has been implicated more frequently (Howell and Stipanovic 1979; Kloepper et al. 1980; Weller and Cook 1983; Kloepper et al. 1988; Thomashow and Weller 1988; de Freitas and Germida 1991; McLoughlin et al. 1992). Thus, in some instances, expression of plant growth-promotion is dependent on interactions between the introduced PGPR and indigenous target soil microorganisms. In addition to deleterious or pathogenic microorganisms, however, many non-target rhizosphere inhabitants, considered neutral or beneficial to plant growth, are necessarily subject to interactions with introduced PGPR. The nature of these interactions, either antagonistic, neutral, or synergistic, may play an important role in the subsequent expression of plant growth-promotion attributed to PGPR.

Because arbuscular mycorrhizal fungi (AMF) are a common component of the rhizosphere microflora and generally regarded as beneficial to plant growth, it is logical to consider the effect of interactions between PGPR and AMF on plant growth. Indeed, Paulitz and Linderman (1991) have suggested that it may be necessary to re-evaluate growth promotion attributed to PGPR in terms of probable interactions with AMF. Moreover, they suggested that inconsistent responses to microbial inoculants may reflect both beneficial and deleterious interactions between introduced microorganisms and AMF. Unfortunately, although potential interactions are of considerable importance when evaluating the consequences of using inoculants to enhance plant growth, limited information is available (Paulitz and Linderman 1989).

This study assessed the effect of interactions between *Pseudomonas* spp. and the AMF isolate *G. clarum* NT4 on plant growth of spring wheat. The effect of these pseudomonads on *G. clarum* NT4 spore germination was also investigated.

Materials and methods

AMF inoculant

Spores of *Glomus clarum* (Nicol. and Schenck) isolate NT4 (Talukdar and Germida 1993) were multiplied in pot cultures using corn (*Zea mays* L. var. Golden Bantam) as the host crop and grown for 90 days in a 1:1 (v/v) soil:sand mixture. *Glomus clarum* NT4 is known to promote growth of spring wheat (Talukdar and Germida 1994). Following multiplication, pot cultures were stored at 5 °C. Prior to spore germination experiments, spores were held at 20 °C for 14 days to improve and synchronize spore germination (Safir et al. 1990).

AMF spores were recovered from pot cultures by wet sieving and sucrose density gradient [20:60% sucrose (w/v)] centrifugation (Daniels and Skipper 1982). Spores were collected from the sucrose interface using a Pasteur pipette and thoroughly washed in running tap water. Debris and damaged or discolored spores were discarded. Spores were surface decontaminated in a solution of 5.0% (w/v) chloramine-T (Sigma Chemical Co., St. Louis, MO) and 0.1 mg ml^{-1} sodium dodecyl sulfate (BDH Chemicals, Toronto, ONT) in reverse osmosis water, for 30 min at 30 °C (Tommerup and Kidby 1980). Spores were isolated and decontaminated within 24 h of initiating an experiment and were maintained in tap water at 5 °C during experimental preparation.

Pseudomonad inoculants

Pseudomonas cepacia strains R55 and R85, *P. aeruginosa* strain R80, *P. fluorescens* strain R92, and *P. putida* strain R104 (de Freitas and Germida 1990a) were maintained at 5 °C on King's B medium (King et al. 1954) slants. These pseudomonads are used routinely in our laboratory as model PGPR because they are known to enhance the yield of a variety of plants including winter wheat (de Freitas and Germida 1990a, 1992a, b), cabbage, lettuce and onion (Germida and de Freitas 1994), and because they exhibit antibiosis against phytopathogenic fungi in soil microcosms (de Freitas and Germida 1991). In addition, strain R85 enhances root hair development on root tissue cultures (de Freitas and Germida 1990b). Bacteria were inoculated into 40 ml King's B broth and grown for 48 h on a rotary shaker (110 rev min^{-1}) at 27 °C. Cultures were harvested by centrifugation (15 min at 5000 *g*), washed twice in phosphate-buffered saline, and resuspended in 40 ml sterile tap water, yielding approximately 10^9 colony-forming units (cfu) ml^{-1} bacterial suspension.

Plant growth in Leonard jars

Wheat seeds (*Triticum aestivum* L. cv. Laura) were sterilized in 70% (v/v) ethanol for 2 min and 1.2% (v/v) sodium hypochlorite for 10 min, and rinsed 10 times in sterile tap water. Sterile seeds were aseptically transferred onto 1.5% (w/v) water agar and allowed to germinate, in the dark, at 27 °C. Following germination (96 h) sterile seedlings were aseptically planted in sterile Leonard jar assemblies (Vincent 1970) containing 185 g crushed montmorillonite ("Turface" Applied Industrial Materials Corporation, Deerfield IL) and 1.5 l modified Hoagland's solution no. 2 (Hoagland and Arnon 1938). Hoagland's solution was modified by reducing the P supply from 115 mg ml^{-1} $NH_4H_2PO_4$ to 12 mg ml^{-1}; adding 60 mg ml^{-1} $(NH_4)_2SO_4$; replacing 0.09 mg H_2MoO_4 with 0.10 mg $Na_2MoO_4 \cdot 2\,H_2O$; and adding 8.0 µg ml^{-1} $Co(NO_3)_2 \cdot 6\,H_2O$. The seedling root system, which consisted of a primary root and two early roots, was carefully inserted into a 5-cm-deep hole in the Turface and the coleoptile was allowed to protrude approximately 1 cm. Using a sterile Pasteur pipette, 200 surface sterilized *G. clarum* NT4 spores were transferred into the bottom of the transplant hole. A pseudomonad inoculant (R85, R92, or R104) was then applied to the seedling roots by pipetting 1 ml of the appropriate pseudomonad suspension (approximately 10^9 cfu) into the transplant hole. Control treatments consisted of autoclaved *G. clarum* NT4 spores and/or an autoclaved R104 suspension. Sterile cotton was packed around the base of each emerging coleoptile and the exposed Turface surface was covered with a tinfoil cap in which a small hole had been fashioned, allowing the coleoptile to protrude through. The completed assemblies were held for 48 h at ambient room temperature and lighting to reduce transplant stress. Following this short adjustment period, the assemblies were transferred to a growth chamber with a 16-h photoperiod, day-night temperature of 25–20 °C, relative humidity of 50%, and irradiance at canopy level of 540 µE $m^{-2}s^{-1}$. Plants were harvested at 40 days after planting (DAP).

The experiment was repeated twice. The first experiment used *Glomus* NT4 spores that had been stored for 8 weeks whereas the second used spores which had been stored for 52 weeks. In all other respects, experimental conditions were identical. Similar plant growth response trends were observed for both Leonard jar experiments, although responses associated with 52-week-old AMF spores generally were more pronounced; thus, data presented are limited to the experiment using 52-week-old AMF spores.

Plant growth in nonsterile soil mix

Plants were grown in 15-cm diameter pots containing 2 kg soil mix consisting of a 1:1 (v/v) mixture of soil [Cutknife Orthic Black Chernozem (Udic haploboroll)] and construction grade silica sand (Grade Sil-7) (Sil Silica, Edmonton, AB). Nutrients were supplied to each pot by thoroughly incorporating 100 ml modified half-strength Hoagland's solution no. 2. Growth medium was brought to two-thirds field capacity (33 kPa) through the addition of reverse osmosis water. Four evenly spaced small aluminium foil funnels (4×4 cm) were pushed into the surface of each pot and the entire assembly was then covered with foil and autoclaved at 121 °C and 124 kPa for 60 min to eliminate any indigenous AMF. Chemical properties of the autoclaved soil mix, performed by the Saskatchewan Soil Testing Laboratory, were as follows: pH 7.1 and conductivity 0.7 mS cm^{-1} (determined on a 1:1 soil:water suspension); 27.4 µg NO_3-N g^{-1} and 21.0 µg SO_4-S g^{-1} (calcium chloride extractable); 14.0 µg P g^{-1} and 180 µg K g^{-1} (sodium bicarbonate extractable); 0.60 µg Cu g^{-1}, 9.06 µg Fe g^{-1}, 0.74 µg Zn g^{-1}, and 44.2 µg Mn g^{-1} (diethylene triaminepentaacetic acid extractable).

Soil microorganisms were reintroduced into the autoclaved soil mix by adding 20 ml of a mycorrhizal-free soil filtrate to the surface of each pot. The filtrate was prepared by shaking 50 g soil in 100 ml sterile tap water for 2 h. The supernatant was passed through a graded filter series, ending with a 1.2-µm-pore-size membrane filter. The resulting filtrate contained 10^4 cfu ml^{-1}. Soil was brought to field capacity by adding sterile distilled water, and allowed to incubate at room temperature for 7 days. Following incubation, reinoculated soil contained 10^7 cfu g^{-1} soil.

Surface sterile spring wheat seeds (*Triticum aestivum* L. cv. Laura) were inoculated by immersion in the appropriate pseudomonad suspension (ca. 10^9 cfu ml^{-1}) for 4 h on a rotary shaker (70 rev min^{-1}) at 27 °C. This procedure yielded 10^7–10^8 cfu $seed^{-1}$. Control seeds were immersed in an autoclaved suspension of R104.

One hundred *G. clarum* NT4 spores were placed in the bottom of each funnel and covered with a 1-cm layer of soil mix. A single inoculated seed was placed in each funnel and covered with 2 cm soil mix. The pots were placed in a growth chamber with a 16-h photoperiod, day-night temperature of 25–20 °C, relative humidity of 50%, and irradiance at canopy level of 510 µE m^{-2} s^{-1}. Following germination and emergence, the plants were thinned to two per pot. Thinning was accomplished by removing the entire funnel containing AMF spores and the emerging seedling from the pot. An equivalent amount of sterile soil mix was replaced in the resulting holes. Two pot experiments were conducted. The first experiment had a single harvest at

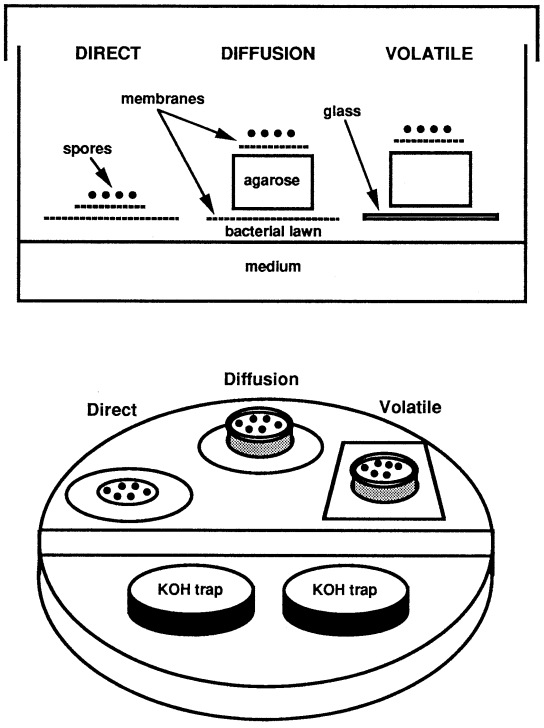

Fig. 1 Schematic drawing of the direct, diffusion and volatile bioassays (not drawn to scale) in cross-view and viewed from above. (Adapted and modified from Liebman and Epstein 1992)

30 DAP. The second experiment was designed to accommodate three destructive harvests at 30, 45 and 78 DAP. Results from the two experiments were similar; thus, only data from the second experiment are presented.

Assays

Shoot and root dry weight, seed yield, plant N and P, and root length (Farrell et al. 1993) were determined. Roots were cleared (Phillips and Hayman 1970), stained (Kormanik et al. 1980) and examined (40 and 100× magnification) for percentage mycorrhizal colonization (Giovannetti and Mosse 1980). Colonized root length was described as the product of root length and percentage colonization.

AMF spore germination

The effects of compounds produced by pseudomonads on *G. clarum* NT4 spore germination were measured using three assays: direct, diffusion, and volatile, which were modifications of those used by Liebman and Epstein (1992) (Fig. 1). The assays were conducted in 15×100-mm sterile polystyrene Petri dishes. Fifteen milliliters of either agarose (1% w/v Agarose 15, BDH Chemicals Ltd., Poole, England) or King's B medium (King et al. 1954) were poured into each Petri dish and allowed to solidify.

Pseudomonad inoculants R85, R92, and R104 were grown (as above) and evenly applied to prepared King's B medium plates using sterile cotton swabs. Inoculated plates were incubated at 27°C for 24 h to establish a bacterial lawn.

The direct assay consisted of placing a sterile 25-mm Microclear polycarbonate 0.22-µm-pore-size membrane (Micron Separations Inc., Westboro, MA) on the medium and/or bacterial lawn surface. Surface-sterilized *G. clarum* NT4 spores were vacuum-filtered onto a second sterile 13-mm Microclear polycarbonate 0.22-µm-pore size membrane and the smaller membrane was placed on the larger membrane. For the diffusion assay, an agarose plug, measuring 14 mm in diameter and 3 mm high, was placed in the center of the larger membrane resting on the media surface and spores on a smaller 13-mm membrane were placed on top of the agarose plug. The volatile assay was similar to the diffusion assay in that spores rested on top of an agarose plug; however, the larger membrane was replaced by a glass coverslip, restricting any direct contact between the bacterial lawn and the agarose plug. In all assays, large membranes, glass coverslips and agarose plugs were incubated on the plates for 24 h before the start of the bioassay. Controls consisted of uninoculated agarose and King's B medium plates. Petri dishes were sealed with Parafilm to confine the internal atmosphere and incubated for 7 days, in the dark, at 27°C.

Two experiments were conducted simultaneously. In the first experiment, the gaseous environment within the sealed Petri dish was allowed to develop undisturbed. In the second experiment, CO_2 was excluded from the gaseous environment using KOH traps. Each KOH trap consisted of 3 ml 1 *M* KOH on a cotton dental roll fitted into a presterilized small inverted bottle lid (2.5×8 mm). Two traps were added to each selected experimental unit (Fig. 1). Concentration of CO_2 in the gaseous phase was determined at the completion of each experiment by inserting a syringe needle, which had been bent to a 90° angle, into the sealed Petri dish and withdrawing a gas sample. The gas sample (1 ml) was analyzed with a gas partitioner (Fisher-Hamilton).

Spore germination and presence of contaminants was assessed at 7 days. Entire plates were discarded if gross contamination was observed. Spores were considered germinated if a germ tube was clearly visible (25–50× magnification).

Experimental design and statistical analyses

A completely randomized design was used for all factorial plant growth experiments. Treatments were replicated 5 times. Analysis of variance and mean separation were performed using the GLM procedure and LSD test in SAS (SAS Institute 1990).

A nested design was used for in vitro spore germination experiments with pseudomonads as main treatments and bioassay (i.e., direct, diffusion and volatile) as subtreatments. Treatments were replicated 5 times; each replicate consisted of a single Petri dish in which each of the direct, diffusion and volatile assays were present. Replicate assays had 14–28 spores. Analysis of variance was performed using the GLM procedure in SAS (SAS Institute 1990). The LSD technique with weighted *t*-values was used to detect differences between subtreatments for different main treatments (Little and Hills 1978).

Results

Plant growth in Leonard jars

Applied in the absence of AMF, both R85 and R92 enhanced shoot and root growth as compared to the uninoculated control (Table 1). For example, root dry weight enhancement resulting from pseudomonad inoculation ranged from 82 to 137% whereas root length was enhanced by as much as 288% as compared to the uninoculated control. When either inoculant was applied in combination with AMF, the magnitude of response was reduced such that R85 no longer resulted in a positive growth enhancement of either shoot or root. In contrast, R104 did not enhance shoot dry matter in the absence of AMF whereas in combination with AMF, a positive shoot dry matter response to R104 was observed. Application of AMF in combination with the pseudomonad inoculants did not result in any additional increases in either dry weight or length of roots

Table 1 Effect of AMF (*Glomus clarum* NT4) and/or *Pseudomonas* sp. (strains R85, R92 and R104) on dry matter production and root length of wheat 40 DAP in Leonard jars

Treatment	Dry weight (g pot^{-1})		Root length (m pot^{-1})
	Shoot	Root	
Control (–AMF)	2.84	1.06	14.1
R85	3.80	2.09	45.6
R92	4.22	2.51	54.7
R104	3.30	1.93	42.1
+AMF	2.94	1.79	34.4
R85+AMF	3.59	1.73	43.4
R92+AMF	4.13	2.45	48.6
R104+AMF	3.94	1.82	41.6
LSD ($P \leqslant 0.05$)	0.86	0.59	10.9

Table 2 Effect of AMF (*Glomus clarum* NT4) and/or *Pseudomonas* sp. (strains R85, R92 and R104) on dry matter production and seed yield of wheat 45 and 78 DAP in nonsterile soil mix

Treatment	Shoot (g pot^{-1})		Root (g pot^{-1})		Seed yield (g pot^{-1})
	45 DAP	78 DAP	45 DAP	78 DAP	
Control (–AMF)	6.6	12.4	4.8	3.6	5.3
R85	6.2	11.6	5.9	3.5	4.8
R92	5.8	11.8	6.8	3.8	4.9
R104	5.0	10.4	4.6	3.0	4.3
+AMF	6.2	11.3	6.6	3.2	4.8
R85+AMF	6.0	10.9	5.2	3.3	4.6
R92+AMF	5.9	11.6	6.4	2.8	4.9
R104+AMF	5.8	11.0	5.8	3.3	4.7
LSD ($P \leqslant 0.05$)	0.8	0.8	1.4	0.7	0.4

beyond that attributable to the pseudomonads alone. In the absence of the pseudomonads, however, AMF significantly enhanced root dry weight and root length as compared to the uninoculated control.

Microscopic examination of roots harvested at 40 DAP revealed evidence of early AMF establishment in AMF-inoculated treatments, including the presence of external and internal hyphae, and appressoria. Colonization was, however, both limited and sporadic, limiting the usefulness of quantitative determinations of AMF colonization.

Concentration of N and P in shoot and root tissue was not significantly affected by either the pseudomonad inoculants or AMF or any of the combined inoculants (data not shown).

Table 3 Effect of AMF (*Glomus clarum* NT4) and/or *Pseudomonas* sp. (strains R85, R92 and R104) on AMF colonization and colonized root length of wheat 78 DAP in nonsterile soil mix

Treatment	AMF colonization (%)	Colonized root length (m pot^{-1})
Control (–AMF)	0	0
R85	0	0
R92	0	0
R104	0	0
+AMF	34.4	308
R85+AMF	20.4	187
R92+AMF	37.8	375
R104+AMF	31.4	286
LSD ($P \leqslant 0.05$)	7.3	109

Plant growth in nonsterile soil mix

Although spring wheat grown in a gnotobiotic Leonard jar system responded positively to pseudomonad inoculants, responses either were undetectable or were negative when the same inoculants were applied in a nonsterile system (Table 2). For example, the greatest shoot and seed yield production typically was achieved by the control plants, although reductions associated with microbial inoculants were not significant in all instances. Only R92 was observed to significantly enhance root growth at 45 DAP. The effect of microbial inoculation on root dry weight was obscured at 78 DAP, likely due to sloughing and aging of the roots, which resulted in relatively high losses during the root extraction procedure.

Shoot dry weight was either unaffected or reduced by AMF alone as compared to the uninoculated control, whereas root dry weight at 45 DAP was significantly enhanced (Table 2). Data indicate the occurrence of interactions between AMF and the pseudomonad inoculants, which influenced the magnitude of responses associated with inoculants applied singly. For example, orthogonal contrast analysis detected significant interactions ($P \leq 0.05$) influencing root dry weight between AMF and both R85 and R92 at 45 DAP. Specifically, when applied as a single inoculant, AMF elicited a positive root growth response as compared to the uninoculated control. In combination with either R85 or R92, however, the magnitude of response was limited.

Seed yield was reduced by *G. clarum* NT4 alone as compared to the uninoculated control (Table 2). Similarly, both R85 and R104 reduced seed yield in the absence of AMF. Application of the pseudomonad inoculants in combination with AMF did not affect seed yield beyond the responses elicited by the inoculants applied singly, indicating that the simple effects of these inoculants were not additive.

Only plants inoculated with *G. clarum* NT4 spores developed detectable AMF colonization. Both AMF colonization and colonized root length were reduced in the presence of R85 (Table 3). AMF colonization was unaffected by the remaining pseudomonad inoculants.

Effect of pseudomonads on AMF spore germination

Generally, germination frequency of *G. clarum* NT4 spores was low and did not exceed 30% (Table 4). Approximately 9% of the AMF spores remained contaminated following surface sterilization as determined on one-tenth strength TSA. There was no visual evidence, however, of contaminant growth from spores placed on agarose. Growth of spore contaminants was observed, however, on some spores subject to direct assays on King's B medium. Entire replicate plates were discarded if gross

Table 4 Effect of *Pseudomonas* sp. (strains R85, R92 and R104) on germination of AMF (*Glomus clarum* NT4) spores detected using direct, diffusion and volatile bioassays

Treatment	Assay	Germination[a]
Control	Direct	4.93 (24)
	Diffusion	4.85 (23)
	Volatile	4.85 (23)
R85	Direct	1.75 (3)
	Diffusion	2.57 (6)
	Volatile	4.50 (20)
R92	Direct	5.12 (26)
	Diffusion	5.41 (29)
	Volatile	4.94 (24)
R104	Direct	4.10 (16)
	Diffusion	4.08 (16)
	Volatile	5.08 (25)
LSD ($P \leqslant 0.05$)		0.65

[a] Values are means of transformed percentage germination data [i.e., $(\%\ \text{germination} + 1/2)^{1/2}$]
[b] Values in parentheses are weighted means of percentage germination data obtained by "detransforming" means of transformed data back to the original units (i.e., % germination)

contamination was observed. Highly significant ($P \leq 0.01$) effects of the pseudomonads on germination of *G. clarum* NT4 spores were detected (Table 4). Specifically, both R85 and R104 reduced spore germination in direct and diffusion assays as compared to germination on uninoculated King's B medium. The magnitude of the reduction was greatest with R85. There was no evidence of reduced germination associated with the volatile assay. Germination was not significantly affected by R92.

Spore germination was similar in both the presence and absence of a CO_2 trap (data not shown). Analyses of gas samples obtained at the completion of the experiments indicated that although the KOH traps effectively removed CO_2 from uninoculated plates, CO_2 levels as high as 0.1% (±0.1%) remained in plates inoculated with R85. Levels of CO_2 in plates inoculated with both R92 and R104 were reduced to 0.04% (±0.04%) by the inclusion of KOH traps. In an uncontrolled environment, the presence of germinating spores alone slightly elevated CO_2 levels above those typical of normal air (i.e., 0.08±0.2% vs 0.03%, respectively). The presence of R85 elevated CO_2 levels to as high as 3% (±2%), whereas levels were limited to 0.9% (±0.4) and 1% (±0.4%) in the presence of R92 and R104, respectively.

Discussion

Plant growth responses to pseudomonad inoculation were highly variable and depended on the growth conditions imposed. Inconsistent responses to beneficial bacteria frequently are reported (Schroth and Weinhold 1986). Inconsistencies associated with microbial inoculants are not surprising because physical and chemical factors, such as soil texture, pH, nutrient status, moisture, temperature and organic matter content, and biological factors, such as multiple interactions between the introduced bacteria, host crop and soil microflora, may influence the establishment, survival and activity of certain organisms whereas other organisms may remain unaffected (Schroth and Weinhold 1986; Kloepper et al. 1989). Because plant growth parameters were generally enhanced by pseudomonad inoculation alone in Leonard jars whereas in nonsterile pot experiments responses were varied and frequently negative, it is suggested that microbial interactions within the rhizosphere may have played an important role in restricting expression of growth promotion.

The mechanisms of growth promotion of beneficial pseudomonads generally are believed to be related to antagonistic interactions, i.e., antibiosis and competition, which result in exclusion of deleterious or pathogenic organisms from the rhizosphere (Lifshitz et al. 1987). It is particularly interesting that shoot, root and total dry matter production of wheat grown in Leonard jars was significantly enhanced by R85, R92 and R104 in the absence of other organisms (Table 1). De Freitas and Germida (1991) similarly reported that R85 and R104 stimulated winter wheat growth under gnotobiotic conditions in Leonard jars. These observations indicate that mechanisms of growth promotion other than biocontrol, such as phytohormone production, may be attributable to these organisms. This hypothesis is in keeping with the observation that inoculation of canola seeds with a *P. putida* strain (GR12-2) increased root length, root dry weight, shoot length and shoot dry weight, under soil-free, gnotobiotic conditions (Lifshitz et al. 1987).

Apparent AMF colonization of wheat grown in Leonard jars was, at best, both limited and sporadic. Although higher levels were observed in the nonsterile soil mix pot experiment, AMF colonization did not exceed 40% (Table 3). Placement of the AMF spore inoculum as a single point source may have contributed to limited root colonization. Restricted AMF colonization also may have been due, in part, to limited rooting volume in both Leonard jar and pot experiments. Bååth and Hayman (1984) observed reduced AMF colonization of onion roots, a highly mycorrhizal plant, when pot size was reduced or plant densities were increased.

In Leonard jars, *G. clarum* NT4 inoculation alone did not enhance shoot dry matter production (Table 1). Moreover, in pot experiments, inoculation with *G. clarum* NT4 alone was associated with reduced shoot dry matter (78 DAP) and seed yield (Table 2). Although contrary to many reports, reduced plant growth attributable to AMF formation is not unique, and occurs when the carbohydrate allocation to the AMF is not compensated for by improved nutrition (Buwalda and Goh 1982; Hays et al. 1982).

Single degree of freedom contrasts detected significant interactions between *G. clarum* NT4 and the pseudomonad inoculants in a number of instances. The nature of these interactions was variable and they were typically manifested as a change in the magnitude of a plant growth response. For example, applied singly, *G. clarum* NT4, R85 and R104 enhanced root dry weight as compared to the uninoculated control in Leonard jars (Table 1). In combination with

G. clarum NT4, however, pseudomonad inoculants R85 and R104 did not enhance root growth, indicating that there was no additive effect of co-inoculation. In contrast, R104 in nonsterile soil reduced shoot and seed yields (Table 2). In combination with *G. clarum* NT4, however, the deleterious effects of R104 were largely overcome and yields were not significantly different than those achieved when plants were inoculated with *G. clarum* NT4 alone. Thus, the interaction between *G. clarum* NT4 and R104 resulted in a change in the magnitude of response to the inoculants applied singly; however, uninoculated controls remained the highest yielding treatment.

The mechanisms by which interactions between *G. clarum* NT4 and the pseudomonad inoculants occurred is not known. However, all of the pseudomonads tested are known to exhibit antibiosis against some plant pathogenic fungal isolates in vitro (de Freitas and Germida 1990a). Although the nature of the antibiosis activity exhibited by these bacteria is not known, de Freitas and Germida (1990a, 1991) suggested that production of both siderophores and antibiotics may play a role.

Paulitz and Linderman (1989) hypothesized that biocontrol agents such as fluorescent pseudomonads might antagonize not only fungal pathogens but also mycorrhizal fungi through production of antifungal compounds or siderophores. Studying interactions between pseudomonads *P. putida* (strains A12, N1R, and R-20) and *P. fluorescens* (strains 2-79 and 3871), and *G. intraradices* and *G. etunicatum*, they observed that strains 3871 and 2-79, which are antibiotic producers, delayed germination of *G. etunicatum* in soil. This effect was undetectable after 7 days, however, and none of the pseudomonads had any detectable effect on subsequent AMF colonization of cucumber roots. They concluded that fluorescent pseudomonads exhibiting biocontrol properties might be compatible with mycorrhizal fungi. Linderman et al. (1991) similarly observed that although spore germination was delayed slightly, seed treatment of pepper and onion with bacterial biocontrol agents had no significant effect on root colonization and plant growth enhancement. These observations led Linderman (1991) to suggest that mycorrhizal fungi may have evolved a tolerance to antifungal substances produced by some soil microorganisms, setting AMF apart from fungal pathogens.

In the present study, germination of *G. clarum* NT4 spores was inhibited when incubated either on membranes placed directly on bacterial lawns of R85 and R104 (i.e., direct assay), or on agarose blocks separated from the bacteria by membranes (i.e., diffusion assay) (Table 4). When the agarose blocks were physically separated from the pseudomonad (i.e., volatile assay), there was no evidence of inhibition. These observations suggest the involvement of a nonvolatile, diffusible substance or substances inhibitory to *G. clarum* NT4 spore germination. It is possible that substances inhibitory to pathogenic fungi known to be produced by these pseudomonads (de Freitas and Germida 1990a) also were inhibitory to *G. clarum* NT4.

Interpretation of the significance of reduced *G. clarum* NT4 spore germination is complicated by plant growth responses to the fungal endophyte which varied from detrimental to beneficial. Reduced AMF spore germination could enhance plant growth if the fungal endophyte acts as a deleterious sink whereas plant growth response could be reduced if the fungal association is beneficial. Dhillion (1992) reported that inoculation of rice plants with a fluorescent *Pseudomonas* sp. reduced mycorrhizal colonization and colonized root lengths of rice. However, dual inoculated plants had greater biomass and higher nutrient levels than plants inoculated with AMF alone. The author hypothesized that because the *Pseudomonas* sp. suppressed AMF colonization, the photosynthate loss to the mycorrhizal associates was subsequently reduced.

We observed that germination of *G. clarum* NT4 spores (Table 4), AMF colonization and colonized root length of wheat roots (Table 3) were significantly reduced by R85. Moreover, interactions between *G. clarum* NT4 and R85 typically altered the magnitude of the observed responses to either inoculant applied alone. Although R104 similarly limited germination of *G. clarum* NT4 spores, colonization of wheat roots was unaffected. These observations suggest that interactions between fungal endophyte, bacteria and host plant, which determine plant growth response, are highly complex. Furthermore, interactions affecting a particular growth stage of the fungal endophyte may not be predictive of the eventual outcome of the association.

The mechanisms by which *G. clarum* NT4 limited the deleterious effects of pseudomonad inoculants on plant growth similarly are not known. However, it is well established that AMF can reduce soil-borne disease or limit disease symptoms (Paulitz and Linderman 1991). The effects of AMF on pathogen and disease development typically are attributed to better nutrition, enhanced plant growth and physiological stimulation of mycorrhizal plants (Dehne 1982). Mechanisms by which disease severity is limited in mycorrhizal plants similarly may have limited the deleterious responses which some of the pseudomonads used in this study occasionally elicited.

Our study demonstrates the importance of evaluating potential growth-promoting microorganisms under a variety of experimental conditions. Plant growth responses attributable to both *G. clarum* NT4 and pseudomonad inoculants were highly varied, apparently due to variations in the physical, chemical and biological conditions imposed in the different experimental regimes. Of particular importance is the evaluation of interactions between microbial inoculants and indigenous microorganisms. Clearly, application of bacterial inoculants would be self-defeating if plant productivity was compromised by antagonistic interactions between the inoculant and potentially beneficial soil microorganisms, such as AMF. Results from this study demonstrate the potential for such negative interactions. Although the mechanisms by which antagonistic interactions occur are not known, early fungal events, such as spore germination, may be inhibited by some pseudomonads. We observed that plant growth responses to inoculation with AMF and pseudomonads typically were not additive in co-inoculated plants. Moreover, a nonvolatile, diffusible substance or substances

produced by strains R 85 and R 104 was inhibitory to *G. clarum* NT 4 spore germination. These observations suggest that substances produced by some pseudomonad inoculants, implicated in the biocontrol of pathogenic fungi, similarly may antagonize AMF.

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