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Plant growth-promoting rhizobacteria alter rooting patterns and arbuscular mycorrhizal fungi colonization of field-grown spring wheat

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Abstract The impact of plant growth-promoting rhizobacteria (PGPR) inoculants on the growth, yield and interactions of spring wheat with arbuscular mycorrhizal fungi (AMF) was assessed in field studies. The pseudomonad inoculants *P. cepacia* R55, R85, *P. aeruginosa* R80, *P. fluorescens* R92 and *P. putida* R104, which enhance growth and yield of winter wheat, were applied at a rate of ca. 10^7–10^8 cfu seed^{-1} and plots established on pea stubble or summer fallow at two different sites in Saskatchewan. Plant shoot and root biomass, yield and AMF colonization were determined at four intervals. Plant growth responses were variable and dependent on the inoculant strain, harvest date and growth parameter evaluated. Significant increases or decreases were measured at different intervals but these were usually transient and final seed yield was not significantly affected. Harvest index was consistently increased by all pseudomonad inoculants; responses to strain R55 and R104 were significant. Root biomass to 60 cm depth was not significantly affected by inoculants except strain R104, which significantly reduced root dry weight. However, root distribution, root length and AMF colonization of roots within the soil profile to 60 cm were significantly altered by inoculants. Most of these responses were reductions in the assessed parameter and occurred at depths below 15 cm; however, strains R85 and R92 significantly increased root dry weight in the 0- to 15-cm zone. These results indicate that some PGPR inoculants may adversely affect mutualistic associations between plants and indigenous soil microorganisms, and suggest a possible reason as to why spring wheat growth was not consistently enhanced by these pseudomonad PGPR.

Key words Pseudomonads · PGPR · AM fungi · Spring wheat · Root systems

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Introduction

Many rhizobacteria demonstrate the ability to promote plant growth under controlled, growth chamber conditions. These plant growth-promoting rhizobacteria (PGPR) typically produce substances that stimulate root growth or inhibit root phytopathogens (Kloepper and Schroth 1981; Kloepper et al. 1989; Thomashow et al. 1990; Hamdan et al. 1991). Unfortunately, the beneficial effects attributable to PGPR under controlled experimental conditions may not reflect their performance in field experiments (Schroth and Weinhold 1986; Kloepper et al. 1988a; de Freitas and Germida 1992b). In fact, the inconsistent response of field-grown crops to PGPR has limited commercial development of these bacteria as crop inoculants.

In natural systems, the behavior of introduced bacterial inoculants (e.g., PGPR) and the subsequent expression of plant growth promotion represents a complex set of multiple interactions between introduced bacteria, associated crops and indigenous soil microflora. These interactions are, in turn, influenced by multiple environmental variables such as soil type, nutrition, moisture and temperature (Kloepper et al. 1989; Glick 1995). Thus, the ability of a bacterial inoculant to promote plant growth can only be fully evaluated when they are tested in association with all of the components of the rhizosphere (Schroth and Weinhold 1986). Similarly, biological interactions between a bacterial inoculant and a particular component of the rhizosphere, such as arbuscular mycorrhizal fungi (AMF), should be regarded as the cumulative effect of all rhizosphere components (Pacovsky et al. 1985; Linderman 1988). Unfortunately, little is known about such interactions, and there is a need to evaluate the impact of potential PGPR inoculants on the natural, beneficial associations between plants and indigenous soil organisms.

Recent work in our lab identified a number of rhizobacteria as PGPR for winter wheat grown under growth chamber and field conditions (de Freitas and Germida 1990a, 1992a,b). These bacteria exhibit a number of traits associated with plant growth promotion including: production of hormones and stimulation of root development (de

Freitas and Germida 1990b), alteration of plant fertilizer use efficiency (de Freitas and Germida 1992a; Germida and de Freitas 1994) and production of compounds inhibitory to phytopathogenic fungi (de Freitas and Germida 1990a, 1991). Although, some of these PGPR promote the growth of other crops besides winter wheat, such as cabbage and lettuce (Germida and de Freitas 1994), the response of field-grown spring wheat to antibiotic-resistant mutants of some of these PGPR is limited even though the inoculants colonize and persist in the rhizosphere over the summer (de Freitas et al. 1994, unpublished results). The possibility that these PGPR may influence the interactions of AMF with spring wheat was assessed in this study.

Materials and methods

Bacterial inoculants

The PGPR inoculants studied were *Pseudomonas cepacia* R55 and R85, *P. aeruginosa* R80, *P. fluorescens* R92 and *P. putida* R104 (de Freitas and Germida 1990a). Working cultures were maintained on King's B (KB) (King et al. 1954) agar (15 g l^{-1}) slants at 5°C.

Bacteria were grown as dense lawns on KB plates for 48 h at 25°C. Cells were scraped from six 150×15-mm plates and resuspended in 370 ml sterile tap water, yielding ca. 10^9–10^{10} cfu ml^{-1}, as determined by spread plate counts on KB agar medium. Bacterial suspensions were poured over 1000 g spring wheat seed in 9.5-l sterile polypropylene trays (36×31×15 cm), and the seed was thoroughly mixed to ensure complete coverage. Seed was soaked in the bacterial suspension for 4 h and stirred hourly. Following incubation, 20 ml 1.5% (w/v) methyl cellulose was added to the seed as a sticker agent. Fine-powder (350 g), acid-purified talc (BDH, Inc.) was then incorporated. Small amounts of additional talc were added, as required, until seeds were thoroughly coated with talc and did not stick to one another. Coated seed was air dried for 12 h, transferred to polyethylene bags and stored at 5°C until seeding. Seeding was accomplished within 24 h of seed treatment. This procedure yielded between 10^7 and 10^8 cfu $seed^{-1}$, as determined by spread plate counts on KB agar medium supplemented with antibiotics (5 mg chloramphenicol, 75 mg cycloheximide, 45 mg novobiocin and 75000 units penicillin G l^{-1}) (Sands and Rovira 1970) immediately prior to seeding. The control treatment consisted of seeds inoculated with an autoclaved suspension of strain R104.

Field experiments

Field experiments were conducted on pea stubble and summer fallow at Hagen and Aberdeen, respectively. Spring wheat (*Triticum aestivum* L. cv. Laura) served as the test crop. Soil at the Hagen site was on Orthic Black Chernozem (Udic haploboroll) of the Cutknife association. Soil at the Aberdeen site was an Orthic Dark Brown Chernozem (Vertic haploboroll) of the Sutherland association. Chemical and physical characteristics of the study soils are given in Table 1.

The study was conducted using a randomized complete block design and consisted of five bacterial treatments (R55, R80, R85, R92, R104) and a control, replicated 6 times. Treated seeds were packed on ice and transferred to the field in coolers until immediately before the seeding operation began.

Seeding at 75 kg ha^{-1} was accomplished using a small-plot research V-belt seed drill (Fabro Ltd., Swift Current, Saskatchewan). Treatment plots were 10×1.05 m in size and contained six seed rows at 17.5-cm spacings. A 60-cm strip was left uncropped between each treatment plot to minimize cross-contamination. To further limit cross-contamination during seeding, seeder V-belts and carrier tubes from the V-belt to the double-disk opener were saturated with 95% ethyl alcohol between treatments and allowed to air dry.

Treatment plots were sampled at 28, 49 and 70 days after planting (DAP), which corresponded to stage 5, stage 10 and stage 10.5 of Feekes scale (Large 1954). Shoots were harvested from a single 1-m row, and subsequently oven dried at 65°C for 72 h. A final harvest at maturity was conducted 99 and 100 DAP at Aberdeen and Hagen, respectively. Four 2-m rows were harvested from each treatment plot. Samples were air dried, threshed and weighed. Representative seed and straw samples were oven dried at 65°C for 72 h and bulk sample weights were adjusted to reflect oven-dry weights. Harvest index was calculated as the percentage ratio of seed mass:seed plus shoot mass.

At each harvest, representative root samples were obtained from within sample rows by excavating the soil from around the seed row to a depth of 15 cm. Roots were extracted from the bulk samples and subsequently assayed for AMF colonization. To facilitate extraction of roots from soil, bulk soil-root samples were frozen and then immersed in water for 30–60 min. Roots were washed free from soil using a gentle stream of water directed through a sprinkler head.

Root biomass was also determined at the Hagen location 56 DAP, which corresponded to stage 10.2 of Feekes scale. Undisturbed soil cores (10.2 cm diameter) were taken from directly within the seed row. In plots underlain by a hard pan unpenetrable with the large corer, two 6.6-diameter soil cores were taken and bulked. Soil cores, taken to a depth of 60 cm, were cut into 15-cm sections. Each section was placed in a plastic bag and frozen. Care was exercised in extracting root samples, in their entirety, as previously described. Root samples were oven dried at 65°C for 72 h and weighed.

Assays

Root lengths of field-grown wheat plants were determined on oven-dry samples extracted from soil-root cores collected from the Hagen site at 56 DAP. Bulk root samples were quartered and representative subsamples (ca. 1 cm in length) were obtained from each quarter. Weight of oven-dry root subsamples varied from 15 to 20 mg. Root subsamples were immersed in water and a single drop of methyl violet stain (1% wt/v in ethyl alcohol) was added, and the subsamples were allowed to soak in the staining solution for 24 h. Root subsamples were prepared as previously described (Farrell et al. 1993) and

Table 1 Physical and chemical characteristics of the Aberdeen and Hagen field soils as determined by the Saskatchewan Soil Testing Laboratory (*P* Olsen-P, *ND* not determined)

Site	Depth (cm)	Texture	OM (%)	NO_3-N (μg g^{-1})	P (μg g^{-1})	K (μg g^{-1})	SO_4-S (μg g^{-1})	Cu (μg g^{-1})	Fe (μg g^{-1})	Zn (μg g^{-1})	Mn (μg g^{-1})	pH	Conductivity (mS cm^{-1})
Aberdeen	0–15	Clay	3.8	18.7	20.0	410	3.8	2.90	25.6	0.74	9.30	7.4	0.7
	15–30	Clay	ND	20.3	14.2	405	2.5	ND	ND	ND	ND	7.4	0.7
	30–60	Clay	ND	29.8	ND	ND	4.2	ND	ND	ND	ND	7.6	0.8
Hagen	0–15	Sandy loam	5.6	19.2	7.5	165	7.2	0.88	24.2	0.68	9.90	7.3	0.3
	15–30	Loam	ND	15.5	7.5	165	4.9	ND	ND	ND	ND	7.4	0.3
	30–60	Loam	ND	11.3	ND	ND	5.9	ND	ND	ND	ND	7.9	0.4

Table 2 Shoot dry weight of spring wheat inoculated with various PGPR inoculants at the Aberdeen and Hagen field sites

Treatment	Shoot dry weight (kg ha^{-1})							
	Aberdeen				Hagen			
	28 DAP	49 DAP	70 DAP	99 DAP[a]	28 DAP	49 DAP	70 DAP	100 DAP[a]
Control	208	1822	3246	5736	274	1912	5596	9976
R55	178	1800	4292**	5401	208	2124	4958	9053**
R80	188	1692	4096**	6010	240	1516	4430*	9728
R85	228	1776	3514	5624	232	2300	4566*	8990**
R92	180	1804	3838*	5532	256	1904	4880	9462
R104	184	1768	4222**	5538	226	1950	4924	8742**
LSD ($P\leq0.1$)	NS[b]	NS	584	NS	NS	574	998	709
LSD ($P\leq0.05$)	NS	NS	704	NS	NS	692	1204	855

*, ** Significantly different from the control at $P\leq0.1$ and $P\leq0.05$, respectively
[a] Weight at harvest includes shoot plus seed
[b] ANOVA F value not significant

root lengths were estimated using the AgVision image analysis system (Decagon Devices, Pullman, Wash., USA).

Percentage mycorrhizal colonization was determined. Root subsamples were cleared (Philips and Hayman 1970) and stained in a 0.05% (wt/v) trypan blue lactic acid solution (Kormanik et al. 1980). Colonization was assessed using a modification of the method proposed by Giovannetti and Mosse (1980). The modified method consisted of placing stained root samples in a 9-cm petri dish marked with a 1-cm grid. Grid lines were scanned using a light microscope (×100 magnification) and the presence or absence of AMF colonization was evaluated at 100 intersects. Colonized root length was described as the product of root length and percentage colonization.

Plant tissue was dried at 65°C for 72 h and weighed. Concentrations of N and P in the plant tissue were determined (Thomas et al. 1967).

Statistical analyses

Analysis of variance of field data was performed using the ANOVA procedure in SAS (SAS Institute Inc. 1990). Mean separation was performed using the LSD test (equivalent to Fisher's least significant difference test) in SAS (SAS Institute Inc. 1990). Where the range of percentage data (i.e., percentage colonization) was between 0% and 20% or 80% and 100%, a square root transformation was used, whereas an inverse sine transformation was used when the percentage data covered a wide range range of values (Steel and Torrie 1980). Because LSD rankings were unchanged by transformation, statistical analyses are reported for untransformed data.

Results

Plant growth responses

Responses of field-grown spring wheat to PGPR inoculants were variable and depended on inoculant strain, harvest date and plant growth parameter evaluated. Moreover, responses differed between experimental field sites and were usually transient. For example, shoot dry weight, determined at 70 DAP, was enhanced by PGPR inoculants R55, R80, R92 and R104 at the Aberdeen field site although no increases in shoot weight were observed at the final harvest (Table 2). In contrast, these PGPR and strain R85 reduced shoot dry weight at the Hagen site (Table 2).

Table 3 Seed yield and harvest index of spring wheat inoculated with various PGPR inoculants at the Aberdeen and Hagen field sites

Treatment	Seed yield (kg ha^{-1})		Harvest index (%)	
	Aberdeen	Hagen	Aberdeen	Hagen
Control	1903	3584	33.5	36.0
R55	1868	3552	35.2	39.4**
R80	2058	3686	34.4	37.6
R85	2000	3386	36.0	37.8
R92	1959	3557	35.9	37.8
R104	1903	3379	34.5	38.7**
LSD ($P\leq0.1$)	NS[a]	NS	NS	1.9
LSD ($P\leq0.05$)	NS	NS	NS	2.3

** Significantly different from the control at $P\leq0.05$
[a] ANOVA F value not significant

Although significant effects of PGPR inoculants on shoot dry weight were observed, seed yield was not significantly affected at either field location (Table 3). Moreover, harvest index was consistently increased by bacterial inoculants although only responses associated with R55 and R104 at Hagen were statistically significant. These results indicate a favorable redistribution of biomass in response to PGPR inoculants; that is, a greater proportion of biomass was allocated to seed yield than to shoot yield in plants inoculated with strains R55 and R104 at Hagen as compared to the control.

None of the PGPR inoculants significantly affected N or P content of shoots at 70 days (Table 4) or seed (Table 5) in a consistent manner at either field site. In some cases increased or decreased N and P accumulation was noted, but these values largely reflected significant changes in shoot biomass.

AMF colonization and root patterns

Colonization of roots (0–15 cm depth) by indigenous AM fungi was generally higher at Hagen than at the Aberdeen field site (Table 6). Application of PGPR inoculants did

Table 4 Nitrogen and P concentration and uptake in the shoots of spring wheat inoculated with various PGPR inoculants at the Aberdeen and Hagen field sites at 70 days

Treatment	Aberdeen				Hagen			
	Nitrogen		Phosphorus		Nitrogen		Phosphorus	
	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}
Control	17.5	56.8	1.9	6.4	14.2	81.8	2.1	12.0
R55	16.6	71.4**	1.8	7.6*	13.3	66.8	1.9	9.8
R80	16.4**	67.4*	1.8	7.6*	16.1	73.2	2.3	10.4
R85	16.0**	55.8	1.7	6.0	11.4	53.2*	1.8	8.6
R92	16.4**	63.0	1.8	6.8	12.0	59.2	1.7	8.4*
R104	16.9	71.2**	1.8	7.6*	12.2	59.6	1.6	8.0*
LSD ($P \leq 0.1$)	0.9	9.4	NS[a]	1.2	3.4	25.2	0.5	3.6
LSD ($P \leq 0.05$)	1.1	11.2	NS	NS	4.1	NS	0.6	NS

*, ** Significantly different from the control at $P \leq 0.1$ and $P \leq 0.05$, respectively
[a] ANOVA F value not significant

Table 5 Nitrogen and P concentration and uptake in the seed of spring wheat inoculated with various PGPR inoculants at the Aberdeen and Hagen field sites

Treatment	Aberdeen				Hagen			
	Seed N		Seed P		Seed N		Seed P	
	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}
Control	27.1	51.5	3.1	5.9	22.9	82.6	3.7	13.4
R55	26.5	49.5	3.2	5.9	22.8	81.3	3.6	12.7
R80	26.3	54.2	3.2	6.5	23.6	87.7	3.7	13.5
R85	26.2	54.5	3.1	6.2	22.0	74.5*	3.7	12.5
R92	28.2	55.1	3.3	6.5	22.9	81.6	3.5	12.5
R104	26.4	50.4	3.1	6.0	22.7	77.1	3.7	12.4
LSD ($P \leq 0.1$)	NS[a]	NS	NS	NS	0.9	7.7	NS	1.0
LSD ($P \leq 0.05$)	NS	NS	NS	NS	1.1	9.3	NS	NS

* Significantly different from the control at $P \leq 0.1$
[a] ANOVA F value not significant

Table 6 AMF colonization of spring wheat roots (0–15 cm depth) inoculated with various PGPR inoculants at the Aberdeen and Hagen field sites

Treatment	AMF colonization (%)							
	Aberdeen				Hagen			
	28 days	49 days	70 days	99 days	28 days	49 days	70 days	100 days
Control	9	34	45	52	32	64	61	72
R55	9	40	42	44	25	54**	57	76
R80	8	43	45	44	27	56*	64	67
R85	9	40	42	41	26	64	65	78
R92	9	43	49	41	21**	51**	61	72
R104	10	41	44	45	22**	60	68	78
LSD ($P \leq 0.1$)	NS[a]	NS	NS	NS	7	7	8	7
LSD ($P \leq 0.05$)	NS	NS	NS	NS	8	9	10	9

*, ** Significantly different from the control at $P \leq 0.1$ and $P \leq 0.05$, respectively
[a] ANOVA F value not significant

not significantly affect AMF colonization at Aberdeen. At Hagen, however, AMF colonization at 28 DAP was reduced by strains R92 and R104, and by R55, R80 and R92 at 49 DAP. Early reductions in AMF colonization were overcome at later harvest dates.

An additional harvest was conducted at Hagen at 56 DAP, at which time both shoot and root biomass were determined. Although data suggest that all PGPR inoculants limited shoot dry weight, only the reduction associated with R55 was statistically significant (Table 7).

Table 7 Shoot and root dry weight, and root/shoot ratios of spring wheat inoculated with various PGPR inoculants and sampled 56 DAP at the Hagen field site

Treatment	Dry weight (kg ha^{-1})		Root/shoot ratio
	Shoot	Root[a]	
Control	3325	3853	1.19
R55	2989*	3190	1.07
R80	3194	3365	1.07
R85	3016	3807	1.28
R92	3219	3828	1.21
R104	3032	2830**	0.94
LSD ($P \leq 0.1$)	324	828	0.32
LSD ($P \leq 0.05$)	NS[b]	999	NS

*, ** Significantly different from the control at $P \leq 0.1$ and $P \leq 0.05$, respectively
[a] Root dry weight to 60 cm depth
[b] ANOVA F value not significant

Root dry weight, to 60 cm, was significantly reduced by application of R104. Moreover, root/shoot ratio was reduced by R104.

Although data indicate that, with the exception of R104, root biomass was not significantly affected by PGPR inoculants, root distribution within the profile to 60 cm was altered (Fig. 1A). For example, although the control and plants inoculated with strain R92 shared nearly identical total root biomass, application of R92 significantly increased root dry weight in the upper 15 cm of the profile and significantly reduced root dry weight at 15–30 cm as compared to the control. Significant redistribution of root biomass as compared to the control similarly was associated with strains R55, R85 and R104.

Length of root in each portion of the profile was also influenced by PGPR inoculation (Fig. 1B). Application of PGPR strains R55, R85, R92 and R104 significantly reduced root length at greater soil profile depths than the control. Root length in the upper portion of the profile was significantly reduced by strain R80, with no concomitant compensatory increases in root length in the lower portions of the profile.

In general, few significant effects of PGPR inoculants on percentage AMF colonization at depth were observed (Fig. 2A). Percentage AMF colonization was enhanced by strain R80 in the lowest portion of the profile whereas strain R92 reduced AMF colonization at the 15- to 30-cm depth. Several significant effects of PGPR inoculants on the length of root colonized by AMF were observed, however, reflecting both nonsignificant trends and statistically significant responses to bacterial inoculation of both percentage AMF colonization and root length (Fig. 2B). Inoculation with strains R55, R85 and R92 reduced AMF-colonized root length in the lower portions of the soil profile. Strain R92 was also associated with a statistically significant increase in AMF-colonized root length in the upper portion of the profile. Application of both R80 and R104 reduced AMF-colonized root length in the upper portion of the profile.

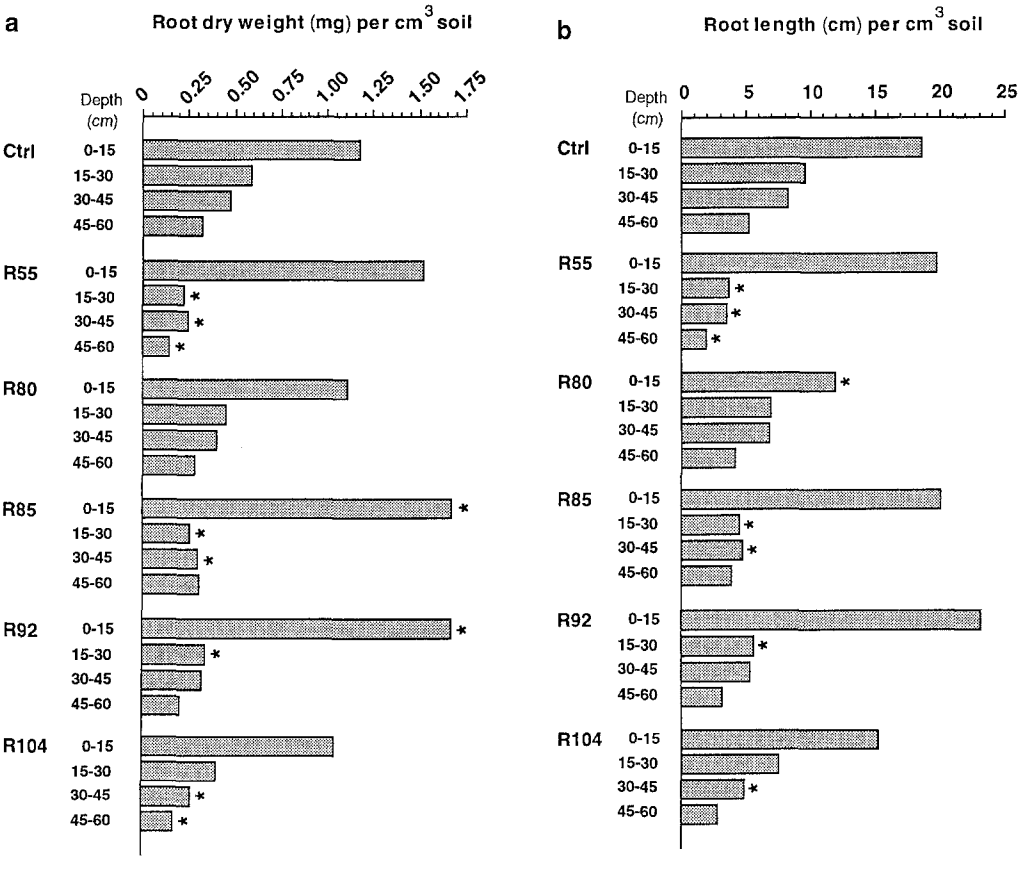

Fig. 1a, b Root dry weight (**a**) and root length (**b**) of spring wheat inoculated with the PGPR *Pseudomonas cepacia* R55, *P. aeruginosa* R80, *P. cepacia* R85, *P. fluorescens* R92 and *P. putida* R104 and sampled 56 days after planting at the Hagen field location. * Indicates a significant difference from the control treatment at the same depth (LSD, $P \leq 0.10$)

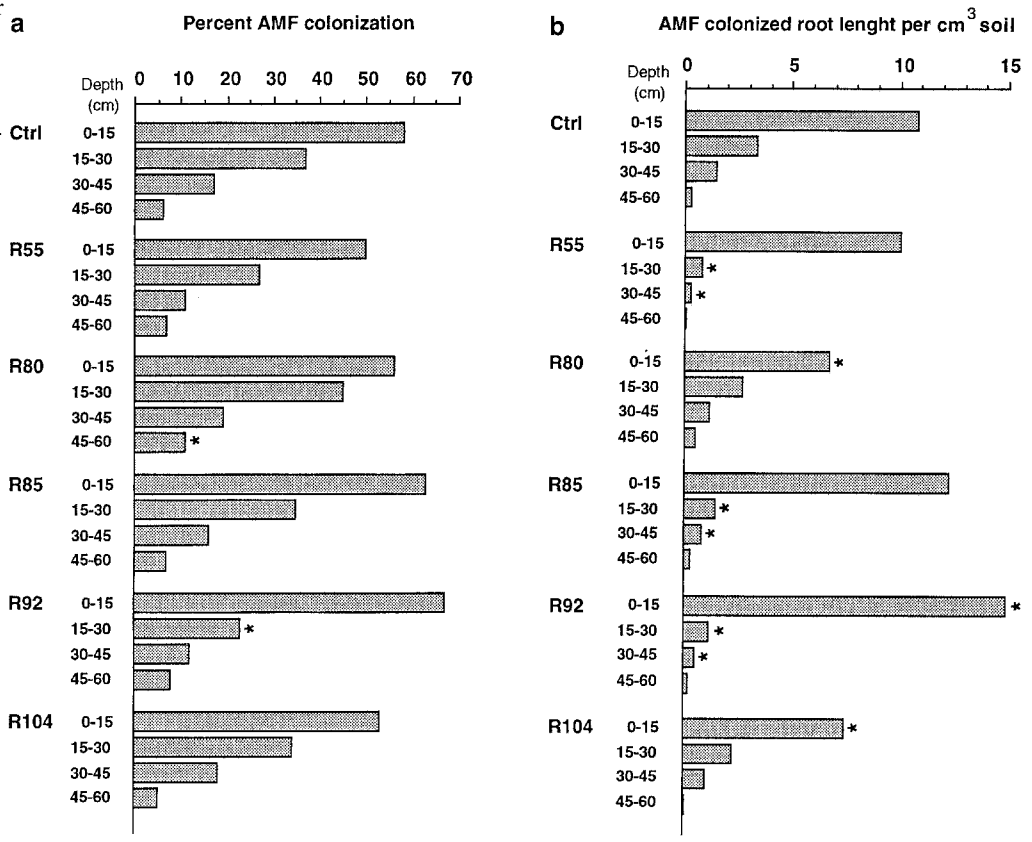

Fig. 2a,b Percentage arbuscular mycorrhizal fungal (*AMF*) colonization (**a**) and AMF-colonized root length (**b**) of spring wheat inoculated with the PGPR *Pseudomonas cepacia* R55, *P. aeruginosa* R80, *P. cepacia* R85, *P. fluorescens* R92 and *P. putida* R104 and sampled 56 days after planting at the Hagen field location. * Indicates a significant difference from the control treatment at the same depth (LSD, $P \leq 0.10$)

Discussion

This field study demonstrated that these PGPR inoculants failed to consistently enhance growth of spring wheat. Moreover, inoculant effects, either beneficial or deleterious, were often transitory and were not consistent from one experiment to the next. In agreement with previous observations (de Freitas and Germida 1990a, 1992b), the plant growth parameters affected by these PGPR also varied, i.e., in some instances shoot growth was influenced whereas in other cases inoculants influenced root growth.

The inability of these bacteria, previously identified as PGPR for winter wheat (de Freitas and Germida 1990a, 1992a,b), to consistently promote spring wheat growth may have been due, in part, to cultivar specificity. Chanway et al. (1988) evaluated the growth promotion of spring wheat by seven strains of *Bacillus*. Originally isolated from the rhizosphere of spring wheat, cv. Katepwa, six isolates promoted root growth of Katepwa in a sterile system but similar increases were not obtained when the test plant was either the genetically similar cv. Neepawa or the more genetically distinct cv. HY320. Additional studies indicated similar specificity both in nonsterile in vitro system and in the field (Chanway and Nelson 1991). These observations led the authors to suggest that a high degree of specificity may exist between plant and bacterial genotypes with respect to the ability of the bacterial inoculant to promote plant growth.

Inconsistent responses to beneficial bacteria are frequently reported (Brown 1974; Broadbent et al. 1977; Schroth and Hancock 1982; Howie and Echandi 1983; Schroth and Weinhold 1986; Schippers et al. 1987; Kloepper et al. 1988a,b; de Freitas and Germida 1990a, 1991, 1992a,b). Moreover, in a compilation of reports of crop yield responses to bacterial inocula in field studies after 1974, Kloepper et al. (1989) noted that in 13 of 26 studies bacterial inocula previously identified as PGPR caused significant yield reductions as compared to the control. It has been suggested that inconsistencies associated with microbial inoculants are not surprising because physical and chemical factors, such as soil texture, pH, nutrient status, moisture and temperature, and organic matter content, and biological interactions in the rhizosphere may affect the establishment, survival and activity of certain organisms whereas other organisms may remain unaffected (Schroth and Weinhold 1986; Kloepper et al. 1989). Thus, apparent discrepancies in experimental results likely reflect differences in experimental conditions including soil and associated indigenous soil microorganisms.

Biological interactions within the rhizosphere extend to interactions with potentially beneficial microorganisms including AM fungi. In our study at Hagen, R104 significantly reduced colonized root length in the top 15 cm of the soil profile by 30.6% as compared to the control, although the reduction in total root length was only 18.7%. In contrast, R92 significantly increased colonized root length in the top 15 cm by 38% although enhance-

ment of total root length was limited to 24%. Thus, our results suggest that the effects of some PGPR inoculants may have been due to interactions between the PGPR and indigenous AM fungi rather than strictly due to direct PGPR-induced plant growth responses. These results are in keeping with our previous lab observations that germination of the AMF *Glomus clarum* NT4 spores, and AMF colonization and colonized root length of spring wheat, were significantly reduced by strain R85, demonstrating the potential for direct interactions between pseudomonad inoculants and AM fungi (Walley 1993). However, some PGPR act synergistically with AMF and enhance AMF colonization and/or plant growth (Pacovsky et al. 1985; Meyer and Linderman 1986; Paulitz and Linderman 1989).

Extraction of wheat roots to 60 cm at Hagen revealed that AMF colonization can extend to a depth of at least 60 cm although the majority of the colonized root length was concentrated within the top 15 cm of soil. Redhead (1977) similarly reported that AM fungi are most abundant in the top 0–15 cm of soil and their numbers decline rapidly below 15 cm. Presence of AM fungi at depth may have resulted from hyphal extension within existing roots or may reflect the presence of AM fungal propagules at depth. An et al. (1990) reported that the vertical distribution of AM fungal propagules closely paralleled root distribution. Thus, although the majority of propagules are usually concentrated in the upper soil profile, a small proportion may occur at considerable depth. Because the upper portion of the soil profile typically is the most biologically active, and frequently has the highest levels of available nutrients, concentration of AMF colonization in this portion of the profile likely confers the greatest benefits to plant growth through improved mineral nutrition. Mycorrhizal colonization at depth may play an important role in water relations, particularly when moisture is limiting.

Because many of the PGPR inoculants tested altered rooting patterns, it seems likely that nutrient and water uptake by the plant roots could be affected. Moreover, altered rooting patterns may affect the development of AMF colonization, indirectly influencing mineral nutrition and water uptake attributable to the fungal endophyte. For example, the significant reduction of AMF-colonized root length in the upper 15 cm of the profile at Hagen due to inoculation with strain R80 was attributable to a concomitant reduction in total root length.

Meyer and Linderman (1986) found that interactions between PGPR and AMF resulted in considerably greater concentrations of some micronutrients (e.g., Fe, Cu, Zn) in shoots of 12-week-old subterranean clover than in plants inoculated with PGPR or AMF alone. Concentrations of N and P were not dramatically affected. We did determine micronutrient concentrations of plant tissue, and there was no clear relationship between inoculant effect on N and P uptake in shoots or seed and the rooting pattern of AMF-colonized root length of field-grown wheat at the Hagen site.

Our results underscore the importance of evaluating potential plant inoculants under a variety of conditions. Schroth and Weinhold (1986) suggested that the erratic nature of plant growth promotion, attributable to bacterial inoculants, can be viewed in two ways. The negative view is that because response to bacterial inoculants is typically erratic, the phenomenon of growth promotion represents a set of interactions too complex to be of practical importance. The positive view is that because growth promotion has been successfully achieved, however erratic, it is clear that considerable potential exists for using microorganisms to promote plant growth. From the latter viewpoint, both positive and negative growth responses can be viewed as clearly indicative of the importance of rhizosphere organisms to plant growth. Moreover, these responses demonstrate the potential to manipulate the rhizosphere population in a manner that significantly influences plant development. Observations which demonstrate direct interactions between applied and indigenous microorganisms further indicate the potential to develop bacterial inoculants which, in addition to promoting plant growth, may interact synergistically with indigenous soil microorganisms. Through elucidation of underlying mechanisms which account for the phenomenon of growth promotion, microbial inoculants can be isolated and developed which not only enhance growth through direct plant growth promotion, but also interact synergistically with beneficial indigenous soil microorganisms such as AMF.

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