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Applied Soil Ecology 31 (2006) 91–100
Applied Soil Ecology
www.elsevier.com/locate/apsoil

Plant growth-promoting rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms

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Received 19 October 2004; accepted 18 March 2005

Abstract

Field trials were conducted in Florida on bell pepper (*Capsicum annuum*) to monitor the population dynamics of two plant growth-promoting rhizobacteria (PGPR) strains (*Bacillus subtilis* strain GBO3 and *Bacillus amyloliquefaciens* strain IN937a) applied in the potting media at seeding and at various times after transplanting to the field during the growing season. In-field drenches of an aqueous bacterial formulation were used for the mid-season applications. The effects of the applied PGPR and application methods on bacterial survival, rhizosphere colonization, plant growth and yield, and selected indigenous rhizosphere microorganisms were assessed. The Gram-positive PGPR applied to the potting media established stable populations in the rhizosphere that persisted throughout the growing season. Additional aqueous applications of PGPR during the growing season did not increase the population size of applied strains compared to treatments only receiving bacteria in the potting media; however, they did increase plant growth compared to the untreated control to varying degrees in both trials. Most treatments also reduced disease incidence in a detached leaf assay, indicating that systemic resistance was induced by the PGPR treatments. However, treatments did not result in increased yield, which was highly variable. Application of the PGPR strains did not adversely affect populations of beneficial indigenous rhizosphere bacteria including fluorescent pseudomonads and siderophore-producing bacterial strains. Treatment with PGPR increased populations of fungi in the rhizosphere but did not result in increased root disease incidence. This fungal response to the PGPR product was likely due to an increase in nonpathogenic chitinolytic fungal strains resulting from the application of chitosan, which is a component of the PGPR formulation applied to the potting media.

Keywords: Bell pepper; Biological control; BioYield™; *Capsicum annuum*; Plant growth-promoting rhizobacteria (PGPR); Soil ecology; Transplants

1. Introduction

Plant growth-promoting rhizobacteria (PGPR) are beneficial native soil bacteria that colonize plant roots and result in increased plant growth (Kloepper, 1994;

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doi:10.1016/j.apsoil.2005.03.007

Glick, 1995; Cleyet-Marcel et al., 2001). While PGPR have been identified within many different bacterial taxa, most commercially developed PGPR are species of *Bacillus* which form endospores that confer population stability during formulation and storage of products. Among the bacilli, strains of *B. subtilis* are the most widely used PGPR due to their disease-reducing and antibiotic producing capabilities when applied as seed treatments (Brannen and Backman, 1993, 1994).

Specific mechanisms involved in pathogen suppression by PGPR vary and include antibiotic production, substrate competition, and induced systemic resistance in the host (Van Loon et al., 1998). Fluorescent pseudomonads are known to suppress soilborne fungal pathogens by producing antifungal metabolites and by sequestering iron in the rhizosphere through the release of iron-chelating siderophores, rendering it unavailable to other organisms (Schippers et al., 1987; Loper, 1988; Paulitz and Loper, 1991; Dwivedi and Johri, 2003). Recent reports by Ryu et al. (2004) have identified several volatile organic compounds produced by a variety of bacteria that promote plant growth and induce systemic resistance in Arabidopsis (*Arabidopsis thaliana*). Beneficial effects of PGPR have also been attributed to shifts in the microbial ecology of the rhizosphere (Kloepper and Schroth, 1981).

Previous research has shown the practicality of introducing PGPR into commercial peat-based substrates for vegetable production in order to increase plant vigor, control root diseases and increase yields (Gagné et al., 1993; Nemec et al., 1996; Kokalis-Burelle et al., 2002a, 2002b, 2003a, 2003b; Kloepper et al., 2004). Results of tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annuum*) trials in Florida included significant increases in tomato and pepper transplant growth during greenhouse production in response to various formulations of PGPR tested (Kokalis-Burelle et al., 2002b). As a result of increased growth, the time required to produce a standard sized transplant was reduced as were greenhouse applications of fertilizer. Also, transplant vigor and survival in the field were improved by PGPR treatments in both tomato and pepper. Trials conducted on muskmelon (*Cucumis melo*) and watermelon (*Citrullus lanatus*) resulted in reduction of root-knot nematode disease severity with several PGPR formulations (Kokalis-Burelle et al., 2003a). PGPR-amended strawberry (*Fragaria ananassa*) plug transplants consistently had higher overall yields compared to nonamended plugs and bare root transplants, with bare-root plants having the lowest yields in 3 years of field studies (Kokalis-Burelle, 2003b). Additionally, under stress or phytotoxic conditions the PGPR-amended plugs performed better than nonamended plugs and bare root transplants (Kokalis-Burelle, 2003b).

Because typical disease control levels observed with PGPR are less than those achieved with chemicals, it is feasible to utilize PGPR as components in integrated management systems that include reduced rates of chemicals and cultural control practices. This approach is becoming increasingly more important as many agricultural chemicals undergo intense scrutiny with regards to their human toxicity and environmental impact. In the United States, the majority of producers of fresh market vegetables and fruits (including tomato, pepper, and strawberry) utilize production systems dependent on methyl bromide soil fumigation. While methyl bromide has contributed significantly to the success of the US vegetable industry, it has been identified as an ozone depleting substance and is currently scheduled to be phased-out of use in industrialized nations by 2005 (WMO, 1998; Federal Register, 2000). Attempts to identify methyl bromide alternatives for vegetable production has led to the re-examination of existing soil fumigants (Gilreath et al., 2001), such as 1,3-D, metam sodium and chloropicrin, development of new broad-spectrum biocides, such as methyl iodide and propargyl bromide (Ohr et al., 1996; Noling and Gilreath, 2001), as well as increasing interest in non-chemical approaches.

It is generally accepted that beneficial microorganisms will not completely replace broad-spectrum fumigants like methyl bromide. However, it has been demonstrated in much of the research described above that these beneficial microorganisms can significantly contribute to plant health and production as components in pest management systems including soil fumigants. In order to successfully integrate biological and chemical approaches it is necessary to more clearly define their effects on rhizosphere ecology under agricultural production conditions and develop dose–response data for establishment of populations

in the rhizosphere. The studies presented here focus on the establishment of the Gram + bacterial isolates in the commercial product BioYield™ (Gustafson LLC, Plano, TX) because this product has consistently improved transplant growth, survival, and disease resistance on a variety of crops in combination with several soil fumigants.

Objectives of this research were to: (1) monitor the population dynamics of two PGPR strains applied as the commercial product BioYield™ to potting media at seeding and as aqueous formulations at various times during the season; (2) determine if supplemental in-field drenches of the liquid bacterial formulation enhance PGPR populations through the season; (3) assess the relationship between population dynamics and plant growth and yield responses; and (4) determine treatment effects on indigenous rhizosphere microorganisms.

2. Materials and methods

2.1. Transplant production

Pepper transplants for both the spring and fall 2001 field trials were produced by USDA personnel in greenhouses at the U.S. Horticultural Research Lab, Ft. Pierce, FL. Bell pepper (*Capsicum annuum*) cultivar Capistrano seeds were planted into 128 cell Styrofoam flats and grown for 4 weeks using overhead irrigation. Plants were fertilized weekly with a solution of Peter's 20-20-20 (N–P–K fertilizer, J.R. Peters Inc., Allentown, PA). Pepper transplant treatments consisted of untreated seedlings and seedlings treated with a chitin-based PGPR formulation containing rifampicin (Rif)-resistant mutants of *Bacillus subtilis* strain GBO3 and *Bacillus amyloliquefaciens* strain IN937a. Rif-resistant mutants were used in all experiments to differentiate between inoculated and indigenous strains of these organisms. These mutants were generated by growing the strains on tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) amended with 100 $\mu g\ ml^{-1}$ Rif (Rif TSA) and selecting colonies with similar growth rates as the wild-type stains. Rif-mutants were then formulated by Gustafson LLC (Plano, TX) in the same manner as the wild-type strains contained in BioYield™. The LS265 liquid formulation of BioYield™ for additional post-plant drench applications during the growing season was prepared using the same Rif-resistant mutants but was formulated without chitin.

2.2. Experimental design

Field trials were conducted at the Uniroyal Chemical Company's Research Station in Sanford, FL. Previous cropping history at the site included 10 years of vegetable production without methyl bromide application, resulting in high levels of plant pathogenic nematodes (primarily *Meloidogyne incognita*) and soilborne fungal pathogens including *Fusarium* spp., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Phytophthora* spp., and *Pythium* spp. For these studies, 2267 $kg\ ha^{-1}$ 8–2–12 fertilizer were broadcast over the field immediately prior to bedding. In order to evaluate treatment effects on rhizosphere microbial populations in a current standard commercial production scenario, soil was fumigated with methyl bromide (67:33 MeBr:pic) at 453 $kg\ ha^{-1}$ 2 weeks before planting. Fumigant was injected into the planting beds during bed formation and black polyethylene mulch was immediately applied to seal beds. Bell pepper seedlings were planted in double rows spaced at 25 cm between plants in a row with 30 plants/row and 60 plants/plot. Plots were 7.6 m long and treatments were replicated eight times. Treatments were: (1) BioYield™ (applied to potting mix according to the label during transplant preparation); (2) BioYield™ + LS265 (liquid suspension formulation without chitin) at transplant; (3) treatment 2 + LS265 drench at 2 weeks after transplanting; (4) treatment 2 + LS265 drench at 2 and 4 weeks after transplanting; and (5) nontreated control. Additional liquid applications of PGPR formulation were included as treatments to determine if they would result in enhanced colonization in the rhizosphere compared to the treatment applied only to the transplant media. In the spring trial, peppers were transplanted into field plots on 17 April. The first LS265 application (treatment 2) was performed on 17 April, with the second and third LS265 applications on 1 May (treatment 4) and 15 May (treatment 5), respectively. Plants were harvested between 15 and 27 June. In the fall trial, peppers were transplanted on 13 September. The first LS265 application (treatment 2) was performed on 13 September, with the second and third LS265 applica-

tions on 27 September (treatment 4) and 11 October (treatment 5), respectively. Plants were harvested between 11 and 15 November. For both trials fertilization was applied throughout the season by drip irrigation according to the Vegetable Production Guide for Florida (Maynard and Hochmuth, 1999).

2.3. Sampling

Sampling for populations followed treatments by 7 days. Sampling times were: (1) before transplanting (untreated, and BioYield™); (2) 7 days after planting (DAP) (untreated, BioYield™, and BioYield™ + LS265 applied at transplanting); (3) 21 DAP (untreated, BioYield™, BioYield™ + LS265 applied at transplanting, and BioYield™ + LS265 applied at 14 DAP); and (4) 35 DAP (all five treatments). Three plants per plot were collected at each sampling time by inserting a trowel approximately 8 cm from the stem, loosening the soil, and extracting the plant and surrounding rhizosphere soil. Destructive samples for population studies during the season were taken from outside a marked harvest zone which would be used at the end of the season to gather yield data. Plants were individually placed in plastic bags and transported in a cooler to the USDA-ARS-USHRL facility for immediate processing. In the lab, residual soil was shaken from each plant, and shoot height, shoot weight, and root weight were measured.

2.4. Microbial isolation

One gram of the roots representing all parts of the root system (tips, lateral roots, and crown) was sampled and ground with clean sterile instruments in 5 ml of sterile phosphate buffer using a Kleco Tissue Pulverizer (Garcia Manufacturing, Visalia, CA). Cylinders were shaken for 5 s and 1 ml of the resulting suspension was placed in 9 ml of sterile phosphate buffer and serially diluted to 10^{-5}, vortexing before each dilution. Fifty microlitres of the appropriate dilution were spread onto two plates of each of the following media using a Model D Spiral Plater (Spiral Systems Inc., Cincinnati, OH): 10% TSA containing rifampicin (100 ppm), 10% TSA, Ohio State medium, Chrome Azurol S (CAS) medium, Richard's medium, Komada's medium, and S1 medium. A nonselective medium (10% TSA) was used for general isolation of culturable bacteria, Ohio State is selective for isolation of sporulating fungi and limits colony growth (Schmitthenner and Williams, 1958), CAS is selective for siderophore producing bacteria (Schwyn and Neilands, 1987), Richard's is selective for *Rhizoctonia* spp. (Martins on and Baker, 1962), Komada's is selective for *Fusarium* spp. (Komada, 1975), and S1 is selective for fluorescent pseudomonads (Gould et al., 1985). Komada, Richards, and Ohio State media were plated at −1 and −2 dilutions while CAS, S1 and 10% TSA were plated at −3, −4 and −5 dilutions, depending on preliminary findings. Samples were then heated to 80 °C for 20 min and the −3 and −4 dilutions were plated on 10% TSA and rif-10% TSA to assess indigenous Gram-positive bacteria and applied Gram-positive bacteria. All plates were incubated at 25 °C in the dark until colonies were countable (2–5 days). Rifampicin resistant biological control bacteria were differentiated from each other by colony morphology.

2.5. Disease challenge

Pepper leaves were removed from plants in all treatments at the end of the season for in vitro experiments to determine induction of resistance against pepper bacterial spot caused by *Xanthomonas vesicatoria*. Detached pepper leaves were placed stem side into 1% water agar medium.

Bacteria were grown on Peptone sucrose agar (20 g/l sucrose, 10 g/l peptone, and 20 g/l agar) for 24 h and suspended in 50 ml sterile tap water containing 10 μl Tween 20. A 20 μl aliquot of 10E7–10E8 bacterial suspension was placed on the leaf halfway between the midrib and leaf margin. The Petri dishes were then sealed with parafilm and placed in an incubator at 27 °C for 5–10 days, or until necrotic lesions appeared. The extent of lesion development within the boundary of each inoculation point was assessed and assigned a rating of 1–5, with 1 = 0–24% chlorosis/necrosis, 2 = 25–49% chlorosis/necrosis, 3 = 50–74% chlorosis/necrosis, 4 = 75–99% chlorosis/necrosis, and 5 = dead leaf.

Incidence of naturally occurring diseases in the field was also assessed. Plant growth measurements including shoot and root weight were taken at harvest. Damage from disease was assessed by performing

subjective root ratings using a 1–5 scale for root condition where 1 = healthy and 5 = 100% necrotic roots. Root galling was assessed using a root gall index based on a scale of 1–10, with one representing no galls and 10 representing severe (100%) galling (Zeck, 1971).

2.6. Yield

Thirty plants were designated in a harvest zone in the center of each plot at the beginning of the season. All destructive samples for population studies during the season were taken from outside of this area. Plots in the spring trial were harvested twice while the fall trial was harvested once because of cold weather at the end of the season. Data are reported as total yield for each season.

2.7. Statistical analysis

Data were statistically analyzed according to standard procedures for analysis of variance (general linear model) and mean separation (least significant difference) (SAS Institute, Cary, NC). All differences referred to in the text were significant at the 5% level of probability.

3. Results

Isolations were performed on roots from BioYield™ treated and control seedlings immediately before transplanting into the field for both spring and fall trials. Both of the bacterial isolates in the BioYield™ formulation were recovered from treated roots at densities of approximately log 3 colony forming units (CFU) (GB03) and log 4 CFU (GB99), and were not recovered from control roots in both trials (data not shown). Populations of other rhizosphere microorganisms including total bacteria did not differ between the BioYield™ and control treatments (data not shown).

In both the spring and fall trials at 7 DAP, the applied strains were detected in treated plots but were not detected at all, or were detected at significantly lower levels in untreated plots (Table 1). Treatments had a small significant effect on the number of total heat tolerant bacteria isolated but did not influence total overall bacteria, indicating a shift in the composition of these communities (Table 1). There was largely no effect of added PGPR on numbers of naturally occurring beneficial bacteria including fluorescent pseudomonads and siderophore-producing bacteria (Table 1). Occasional significant increases were

Table 1
Survival of added PGPR and effects on indigenous rhizosphere microorganisms in log colony forming units in spring and fall field trials in Florida at 7 days after planting

Applied	GB99		GBO3		Total heat-tolerant bacteria		Total bacteria	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Untreated	0.00 b[a]	0.54 b	2.29 b	0.15 b	4.24 b	4.91 c	5.82 a	7.20 a
BioYield™	0.95 a	4.88 a	5.06 a	1.09 a	4.58 a	5.36 b	5.86 a	7.30 a
BioYield™ + LS265 at transplant	0.34 ab	4.95 a	4.94 a	1.43 a	4.37 b	5.73 a	5.69 a	7.26 a
LSD (0.05)	0.70	0.45	0.77	0.91	2.00	0.16	0.40	0.14

Indigenous	Fluorescent pseudomonads		Siderophore producers		*Rhizoctonia*		*Fusarium oxysporum*		Total fungi	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Untreated	1.46 a	5.65 a	2.76 a	6.51 a	2.03 b	1.32 b	0.74 b	1.47 a	3.28 b	3.25 c
BioYield™	1.66 a	5.23 b	2.76 a	6.56 a	2.53 ab	2.31 a	1.08 ab	1.21 a	3.70 a	4.00 a
BioYield™ + LS265 at transplant	1.93 a	5.35 ab	2.89 a	6.51 a	2.60 a	2.59 a	1.65 a	1.32 a	3.72 a	3.77 b
LSD (0.05)	0.53	0.41	2.00	0.14	0.55	0.40	0.67	0.61	0.15	0.17

[a] Means followed by the same letter are not significantly different at $P \geq 0.05$ using analysis of variance (GLM) and mean separation (LSD) (SAS Institute, Cary, NC).

observed in the number of *Rhizoctonia*, *Fusarium*, and total fungi isolated from BioYield™ treatments compared to untreated plots (Table 1). However, increases in fungal populations in the rhizosphere with BioYield™ did not result in increased root disease incidence (data not shown). For both studies, similar trends were observed at 21 DAP, and 35 DAP except with a more pronounced increase of total heat tolerant bacteria and total bacteria isolated from BioYield™ treatments when compared to the untreated control (Tables 2 and 3). In addition, at 35 DAP, there was a significant increase in siderophore producers in all PGPR treatments when compared to the untreated control for both trials (Table 3).

By harvest time at 63 DAP, few differences were evident in indigenous populations with the exception of an increase in *Rhizoctonia* populations with most treatments, while the number of the applied strains remained consistently high in the spring trial (Table 4). In the fall, few effects on indigenous populations were observed and populations of applied strains dropped dramatically to levels that were undetectable (Table 4). The drop in populations of the applied PGPR strains at 63 DAP in the fall may have been due to freezing temperatures which resulted in the loss or decline of plants.

Although increased frequency of post-plant treatments did not increase populations of applied microorganisms, several BioYield™ treatments significantly increased shoot weight, shoot height, and root weight compared to the untreated control (Table 5). In the fall, BioYield™ treatments had greater shoot weight, but did not have a consistent effect on shoot height, or root weight (Table 5). Most treatments also reduced disease incidence in the detached leaf assay in the spring trial compared to the control, while there were no differences in the fall trial (Table 5). Treatments in either trial did not result in increased yield, which was highly variable (Table 5). Yields in the fall experiment were lower than those in the spring experiment due to freezing temperatures in early November during the harvest period, resulting in only one harvest.

Table 2
Survival of added PGPR and effects on indigenous rhizosphere microorganisms in log colony forming units in spring and fall field trials in Florida at 21 days after planting

Applied	GB99		GBO3		Total heat-tolerant bacteria		Total bacteria	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Untreated	0.00 b[a]	1.50 b	0.18 b	0.30 b	5.18 b	5.13 c	6.70 b	7.40 a
BioYield™	3.73 a	4.87 a	2.84 a	3.58 a	5.40 a	5.75 b	6.81 ab	7.65 a
BioYield™ + LS265 at transplant	4.42 a	4.77 a	2.48 a	3.64 a	5.48 a	5.90 b	6.86 a	7.39 a
BioYield™ + LS265 at transplant and 14 DAP	4.25 a	4.89 a	2.57 a	3.71 a	5.41 a	6.20 a	6.76 ab	7.48 a
LSD (0.05)	0.69	0.54	1.01	0.34	0.12	0.22	0.12	0.15

Indigenous	Fluorescent pseudomonads		Siderophore producers		*Rhizoctonia*		*Fusarium oxysporum*		Total fungi	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Untreated	3.79 a	5.37 a	5.60 a	6.51 a	0.95 b	2.35 b	0.64 b	1.23 a	3.44 b	3.12 b
BioYield™	3.54 a	5.23 a	5.67 a	6.36 a	3.12 a	3.42 a	2.17 a	1.28 a	3.84 a	3.91 a
BioYield™ + LS265 at transplant	3.76 a	5.32 a	5.78 a	6.46 a	3.18 a	3.36 a	2.77 a	1.25 a	3.80 a	3.85 a
BioYield™ + LS265 at transplant and 14 DAP	4.09 a	4.98 a	5.58 a	6.44 a	2.82 a	3.59 a	2.60 a	1.48 a	3.76 a	3.76 a
LSD (0.05)	0.85	0.39	0.26	0.23	0.61	0.46	0.60	0.58	0.31	0.39

[a] Means followed by the same letter are not significantly different at $P \geq 0.05$ using analysis of variance (GLM) and mean separation (LSD) (SAS Institute, Cary, NC).

Table 3
Survival of added PGPR and effects on indigenous rhizosphere microorganisms in log colony forming units in spring and fall field trials in Florida at 35 days after planting

Applied	GB99		GBO3		Total heat-tolerant bacteria		Total bacteria	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Untreated	0.90 b[a]	0.83 b	0.00 b	0.15 b	5.29 c	5.08 c	6.55 b	7.55 b
BioYield™	4.27 a	4.13 a	0.31 ab	2.20 a	5.73 b	5.32 b	6.71 a	7.67 a
BioYield™ + LS265 at transplant	4.49 a	4.69 a	0.82 a	2.55 a	5.79 b	5.83 a	6.69 a	7.60 ab
BioYield™ + LS265 at transplant and 14 DAP	4.32 a	4.47 a	0.37 ab	2.43 a	5.64 b	5.84 a	6.75 a	7.66 a
BioYield™ + LS265 at transplant, 14 and 28 DAP	4.57 a	4.66 a	0.31 ab	2.70 a	6.12 a	5.73 a	6.74 a	7.58 ab
LSD (0.05)	0.58	0.58	0.63	0.75	0.32	0.22	0.12	0.11

Indigenous	Fluorescent pseudomonads		Siderophore producers		*Rhizoctonia*		*Fusarium oxysporum*		Total fungi	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Untreated	3.35 ab	5.76 a	4.90 b	6.69 b	0.0	2.47 d	1.14 b	1.65 ab	2.57 b	3.03 c
BioYield™	3.76 ab	5.91 a	5.09 ab	7.02 a	0.0	3.13 c	1.88 a	1.68 ab	3.42 a	3.75 ab
BioYield™ + LS265 at transplant	3.04 b	5.82 a	4.99 b	6.92 ab	0.0	3.66 ab	2.11 a	1.67 ab	3.31 a	3.88 a
BioYield™ + LS265 at transplant and 14 DAP	4.17 a	5.78 a	5.25 a	6.88 ab	0.0	3.23 bc	1.56 ab	1.46 b	3.13 ab	3.56 b
BioYield™ + LS265 at transplant, 14 and 28 DAP	3.44 ab	5.76 a	5.00 b	6.79 ab	0.0	3.72 a	1.89 a	2.03 a	2.84 ab	3.84 a
LSD (0.05)	0.94	0.17	0.24	0.23	0.0	0.44	0.59	0.53	0.58	0.23

[a] Means followed by the same letter are not significantly different at $P \geq 0.05$ using analysis of variance (GLM) and mean separation (LSD) (SAS Institute, Cary, NC).

4. Discussion

Improvements in plant growth and disease resistance to a broad array of plant pests can be accomplished using PGPR (Kloepper et al., 2004). The concept of introducing PGPR into the rhizosphere using the transplant plug is based on the hypothesis that their establishment in the relatively clean environment of the planting media would afford them an opportunity to develop stable populations in the seedling rhizosphere, and that these populations would then persist in the field. It was also hypothesized that early exposure to PGPR might precondition young plants to resist pathogen attack after transplanting in the field. It is well recognized that PGPR can positively influence plant growth and resistance to pathogens (Kloepper, 1994; Glick, 1995; Cleyet-Marcel et al., 2001). However, it is necessary to establish a greater understanding of the dynamics of applied beneficial organisms under field conditions in order to optimize their application methods and timing. It is also important to understand the effects of applied biocontrol strains on populations of indigenous beneficial bacteria including fluorescent pseudomonads, which commonly occur in the rhizosphere, and are known to suppress pathogen establishment and disease (Schippers et al., 1987; Loper, 1988; Paulitz and Loper, 1991; Dwivedi and Johri, 2003).

PGPR strains applied to the potting media as BioYield™ resulted in stable populations of those strains in the pepper rhizosphere that persisted throughout the growing season. Supplemental appli-

Table 4
Survival of added PGPR and effects on indigenous rhizosphere microorganisms in spring and fall field trials in Florida at 63 days after planting (end of season) in log colony forming units

Applied	GB99		GBO3		Total heat-tolerant bacteria		Total bacteria	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Untreated	0.19 c[a]	0.0	0.18 b	0.0	4.97 a	5.13 c	6.25 a	7.36 a
BioYield™	2.18 ab	0.0	0.78 ab	0.0	4.98 a	5.43 b	6.207 a	7.25 a
BioYield™ + LS265at transplant	3.22 a	0.0	1.11 a	0.0	5.11 a	5.67 ab	6.282 a	7.25 a
BioYield™ + LS265 at transplant and 14 DAP	1.76 b	0.0	0.56 ab	0.0	5.23 a	5.57 ab	6.29 a	7.28 a
BioYield™ + LS265 at transplant, 14 and 28 DAP	2.54 ab	0.0	0.77 ab	0.0	5.27 a	5.78 a	6.18 a	7.31 a
LSD (0.05)	1.13		0.87		0.34	0.27	0.18	0.16

Indigenous	Fluorescent pseudomonads		Siderophore producers		*Rhizoctonia*		*Fusarium oxysporum*		Total fungi	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Untreated	4.66 a	6.05 b	5.57 a	6.84 a	2.09 b	2.32 a	1.78 ab	1.80 a[a]	2.54 a	2.86 c
BioYield™	4.80 a	6.18 a	5.39 a	6.64 a	2.64 a	2.51 a	1.90 ab	1.69 a	2.73 a	3.26 a
BioYield™ + LS265 at transplant	4.94 a	6.15 ab	5.52 a	6.71 a	2.66 a	2.73 a	2.15 a	1.67 a	2.79 a	3.13 ab
BioYield™ + LS265 at transplant and 14 DAP	4.79 a	6.15 ab	5.22 a	6.71 a	2.24 ab	2.35 a	1.78 ab	1.75 a	2.55 a	2.99 bc
BioYield™ + LS265 at transplant, 14 and 28 DAP	4.32 a	6.10 ab	5.39 a	6.84 a	2.55 a	2.53 a	2.03 a	1.90 a	2.79 a	3.18 ab
LSD (0.05)	0.68	0.11	0.41	0.23	0.42	0.46	0.54	0.55	0.34	0.21

[a] Means followed by the same letter are not significantly different at $P \geq 0.05$ using analysis of variance (GLM) and mean separation (LSD) (SAS Institute, Cary, NC).

Table 5
Plant growth rating, yield, and disease challenge with pepper bacterial spot (*Xanthomonas vesicatoria*) at 63 days after planting (end of season)

	Shoot weight (g)		Shoot height (cm)		Root weight (g)		Total yield (kg/ha)		Detached leaf rating[b]	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Untreated	326.1 b[a]	47.4 b	34.8 c	33.3 a	18.6 b	11.3 ab	17170 a	5360 a	5.0 a	1.1 a
BioYield™	359.7 ab	59.8 b	35.6 c	34.1 a	20.4 ab	13.6 ab	14122 a	4513 a	3.4 b	1.0 a
BioYield™ + LS265 at transplant	386.3 ab	51.3 b	36.4 abc	33.9 a	23.0 ab	11.5 ab	14212 a	5975 a	4.0 ab	0.8 a
BioYield™ + LS265 at transplant and 14 DAP	420.2 ab	85.3 a	37.1 ab	35.2 a	24.1 ab	14.6 a	15373 a	5080 a	3.6 b	0.7 a
BioYield™ + LS265 at transplant, 14 and 28 DAP	447.2 a	68.1 ab	38.1 a	33.9 a	26.3 a	10.5 b	15111 a	5300 a	3.4 b	0.8 a
LSD (0.05)	100.7	26.9	2.2	3.5	6.1	3.5	3212	2313	1.4	0.6

[a] Means followed by the same letter are not significantly different at $P \geq 0.05$ using analysis of variance (GLM) and mean separation (LSD) (SAS Institute, Cary, NC).

[b] Detached leaf assay rating = 1–4, 1 = 0–24% chlorosis/necrosis, 2 = 25–49% chlorosis/necrosis, 3 = 50–74% chlorosis/necrosis, 4 = 75–100% chlorosis/necrosis.

cation of the PGPR in a liquid formulation during the growing season did not result in increased populations of applied bacteria or increased total bacterial carrying capacity of the rhizosphere, although additional uses did increase plant growth. Also, application of these strains did not adversely affect populations of beneficial indigenous rhizosphere bacteria including fluorescent pseudomonads and siderophore producing bacteria.

The occasional increase in the number of *Rhizoctonia*, *Fusarium*, and total fungi isolated from BioYield™ treatments compared to untreated plots may be a response of those organisms to the chitosan contained in the BioYield™ formulation, which is known to increase populations of chitinolytic microorganisms including fungi (Kokalis-Burelle et al., 1992; Backman et al., 1994). However, none of the organisms isolated from the rhizosphere in our experiments were tested for chitinolytic ability or for pathogenicity to pepper. Because no increase in root disease was observed among treatments in correlation with increases in *Rhizoctonia* and *Fusarium* isolated from soil, it can be interpreted that these fungal isolates were not aggressive pathogens.

Although significant responses occurred in plant shoot weight, shoot height, and root weight with several treatments at the end of the growing season, there was no corresponding significant increase in pepper yield as seen in previous trials (Kokalis-Burelle et al., 2002b). However, there was a significant disease resistance response to inoculation with the pepper bacterial spot pathogen (*Xanthomonas vesicatoria*) with most of the PGPR treatments at the end of the spring season. Increased resistance to bacterial pathogens is an important and valuable effect of treatment with PGPR because bacterial pathogens can be extremely difficult and expensive to control. Also, the reduction in foliar disease is an indication of an induced systemic resistance response to treatment with PGPR that may also confer some level of general resistance to other pathogens. This research demonstrates that pre-conditioning for resistance can be accomplished in high-value transplanted crops by applying PGPR to the transplant plug at seeding, resulting in season-long establishment of stable populations of the applied PGPR in the rhizosphere of pepper that do not detrimentally affect beneficial native rhizosphere microorganisms.

Acknowledgements

This research was supported by a Cooperative Research and Development Agreement between USDA, ARS and Gustafson LLC (CRADA #58-3K95-8-640). PGPR formulation LS213 has been developed into the commercial product BioYield™ (Gustafson LLC, Plano, TX). Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture. The authors would like to acknowledge Donald S. Kenney of Gustafson LLC for continuing support, and Amanda Rinehart, John McEnroy, and Sissy Ivey for technical assistance.

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