



Field evaluation of plant growth-promoting Rhizobacteria amended transplant mixes and soil solarization for tomato and pepper production in Florida

N. Kokalis–Burelle^{1,4}, C. S. Vavrina², E. N. Roskopf¹ & R. A. Shelby³

¹USDA, ARS, U.S. Horticultural Research Lab, Ft. Pierce, FL, USA.

²University of Florida, Immokalee, FL, USA.

³USDA, ARS, Aquatic Animal Health Research Lab, Auburn, AL, USA.

⁴Corresponding author

Received 27 February 2001. Accepted in revised form 19 September 2001

Key words: Methyl bromide, rhizobacteria, root-knot nematode, solarization, transplant amendments, vegetables

Abstract

Field trials were performed in Florida to evaluate tomato and pepper transplants amended with formulations of several plant growth-promoting rhizobacteria (PGPR) in a production system that included soil solarization. Transplants grown in five different formulations of PGPR were planted into plots treated by soil solarization, MeBr fumigation, or untreated soil. Treatments were assessed for incidence of several naturally occurring tomato and pepper pathogens including root-knot nematode (*Meloidogyne incognita*) and species of *Pythium*, *Phytophthora*, and *Fusarium*. Highly significant increases in tomato and pepper transplant growth occurred in response to most formulations of PGPR tested. Transplant vigor and survival in the field were improved by PGPR treatments in both tomato and pepper. Diseases of tomato caused by root-knot nematodes, *Fusarium*, *Phytophthora*, and *Pythium* were not affected by PGPR treatments. PGPR formulation LS261 reduced numbers of root-knot nematode galls on pepper while pepper root condition was improved with formulations LS213, LS256 and LS261. Individual PGPR strains affected the number of *Pythium* colonies isolated from pepper roots, but did not affect isolation of *Pythium* from tomato roots. Greater numbers of colonies of *Pythium* were isolated from pepper roots in the MeBr treatment and fewest in the solarization treatment. Numbers of colony forming units of *Fusarium* were significantly higher in the untreated soil than in MeBr fumigated or solarized soil with no effect of PGPR on isolation of *Fusarium* from either crop. Incidence of wilt symptoms on tomato was significantly lower in MeBr treated plots and highest in the untreated plots. Yield of extra large tomato fruit and total yield increased with PGPR formulation LS256. Yield of pepper was increased with formulations LS255 and LS256. Solarization combined with LS256 on pepper produced yields comparable to MeBr.

Abbreviations: (MeBr) – methyl bromide; (PGPR) – plant growth-promoting rhizobacteria; (LDPE) – low-density polyethylene; (DAP) – days after planting; (CFU) – colony forming units

Introduction

In the United States, the majority of producers of fresh market vegetables and fruits including tomato, pepper, and strawberry utilize production systems that are highly dependent on soil fumigation with methyl bromide (MeBr). Florida is the nation's largest producer of fresh market tomatoes and peppers. Tomato

production in Florida for the 1997–1998 growing season had an estimated value of more than \$473 million, while bell peppers contributed \$272 million to a vegetable industry valued at more than \$1.7 billion (Anonymous, 1997). These industries currently use more than 6000 metric tons of MeBr annually for soil fumigation. Sixty-two percent of this is used in the production of tomatoes and 26% is used in bell pepper

production. While MeBr has contributed significantly to the success of the vegetable industry in Florida, it has been identified as a major ozone depleting substance. The current phase-out schedule requires total elimination of use in industrialized nations by 2005.

Biological control agents have been used successfully in several pathogen/host systems to enhance plant growth and control disease. Several mechanisms have been demonstrated. Biological control organisms can act as antagonists or predators of the targeted pest, or by inducing resistance in the host (Cook and Baker, 1983). Plant growth promoting rhizobacteria (PGPR) have been shown to enhance plant growth and protect roots from invasion by pathogens by a variety of mechanisms including production of antibiotics, hydrogen cyanide, siderophores, and induced systemic resistance (Kloepper et al., 1980; Weller, 1988; Zehnder et al., 2001). Formulation of biocontrol agents using organic amendments, such as by-products from agriculture and other industries, can contribute to the control of soilborne pathogens in a variety of crops by acting as inducers of plant defense responses (Benhamou and Thériault, 1992). The addition of organic matter to soil to control soilborne pathogens and establish beneficial soil microflora is well documented (Hoitink and Fahy, 1986; Kokalis-Burelle et al., 1994), but often impractical to apply in the field on a large scale. One method that addresses this shortcoming is the preinoculation of transplants using amendments to soil-less transplant mixes.

Soil solarization is the process of using clear polyethylene mulch to heat the upper 5–25 cm of soil to temperatures that are detrimental to pathogenic soilborne organisms. Solarization has been successfully implemented in several commercial tomato and pepper trials in the southeastern United States (Chellemi et al., 1993; Chellemi et al., 1997). Soil solarization during the hot summer months is well suited for fall vegetable production in some areas, however, it does not control all soilborne pests (Gilreath et al., 1999). Use of solarization in combination with other strategies to limit pathogen impact and increase yields would improve the viability of this technique for use in Florida vegetable production.

The objectives of this research were to: (1) test selected combinations of PGPR and organic amendments in soil-less mixes for their effect on transplant growth, vigor and survival in the field; (2) examine the combined effects of transplants grown in amended mixes and soil solarization against a wide range of soilborne pests, (3) determine the impact of combin-

ation treatments on yield of tomato and pepper in commercial production systems.

Materials and methods

Transplant production

Field trials were conducted in the fall of 1998 and 1999 at the Uniroyal Chemical Company Inc., Florida Research Station, Sanford, FL. One tomato and one pepper trial were performed each year. Tomato and pepper transplants for the fall 1998 field season were produced by the USDA, in greenhouses at the University of Florida Indian River Research and Education Center in Ft. Pierce, FL. Transplants for the fall 1999 field season were produced by Speedling, Inc., Bushnell, FL. Tomato cultivar 'Solar Set' or pepper cultivar 'Jupiter' were planted into 128 cell flats and grown in a greenhouse for four weeks using overhead irrigation at the University of Florida and sub irrigation at the Speedling facility. Plants at the University of Florida were fertilized weekly with a solution of Peter's 20–20–20. Plants grown at Speedling were fertilized according to their standard transplant production practices for tomato and pepper. In 1998 trials, tomato and pepper transplant treatments consisted of five PGPR formulations: LS213 (*Bacillus subtilis* strain GBO3 + *Bacillus amyloliquefaciens* strain IN937a), LS254 (*Bacillus subtilis* strain GBO3 + *Bacillus pumilus* strain SE34), LS255 (*Bacillus subtilis* strain GBO3 + *Bacillus subtilis* strain IN937b), LS256 (*Bacillus subtilis* strain GBO3 + *Bacillus pumilis* strain INR7), LS261 (*Bacillus subtilis* strain GBO3 + *Bacillus cereus* strain C4) and an untreated control. In 1999, the treatments exhibiting the greatest potential in the 1998 trials were evaluated again: LS213, LS256, LS261, and an untreated control. The powder formulations included chitin, an organic material previously shown to elicit low levels of resistance responses in a variety of crops including tomato (Benhamou and Thériault, 1992; Benhamou et al., 1994). PGPR formulations, provided by Gustafson LLC (Plano, TX), were mixed into Speedling peat-lite potting mix at a rate of 40:1 (v/v) potting mix:PGPR formulation. Seedlings were evaluated at four weeks after seeding for stem length, stem diameter, leaf area, dry stem weight, dry root weight, dry top weight, leaf:stem ratio, root:shoot ratio, and number of true leaves.

Field experimental design

Previous cropping history at the Uniroyal Chemical Company's Research Station in Sanford, FL, included potato in the spring 1998, followed by early summer fallow without weed control. This was preceded by ten years of vegetable production without MeBr 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. Experiments contained split plots with subplots consisting of PGPR treatments. Main plots consisted of three soil treatments arranged in a randomized complete block design with five replications in 1998 and four replications in 1999. Main plot treatments were: (1) Soil solarization using clear, low-density polyethylene film (LDPE); (2) Untreated soil under 30- μ m-thick, coextruded white on black LDPE mulch; (3) MeBr (425 kg/ha broadcast 98:2 MeBr:chloropicrin) applied under 30- μ m-thick, coextruded white on black LDPE mulch. The 98:2 MeBr:chloropicrin concentration was the commercial standard at the time of the studies. Rates of 425 kg/ha were commonly used by growers in Florida with high root-knot nematode and weed populations. During both years, 2267 kg/ha 8–2–12 fertilizer were broadcast over the field immediately prior to bedding. In both 1998 and 1999, soil was solarized for six weeks using clear polyethylene film. After six weeks of solarization the clear plastic film was painted using white latex-based paint mixed at a 6:1 dilution with water and applied through a CO₂ backpack sprayer. The painted plastic was comparable in color and density to the white on black coextruded mulch used in the untreated and MeBr treated plots. In 1998, beds were formed on 1 July, painted on 19 August, and transplants were planted on 1 September. In 1999, beds were formed on 29 June, painted on 18 August and transplants were planted on 2 September. Beds were 36.5 m long, oriented north-south, and formed under optimum soil moisture for bed shaping and to maximize solarization efficacy. Beds were 20 cm in height, and 0.9 m wide and spaced 1.8 m apart. Subplots were 6.1 m long with tomatoes planted in single rows spaced at 45 cm, and bell peppers planted in double rows also spaced at 45 cm. A single central drip irrigation line was laid just below the soil surface (5 cm). Supplemental overhead irrigation was available to insure proper soil moisture at bedding.

Evaluations for effects on disease and yield

Plants were evaluated for growth parameters at transplanting, survival at 10 days after planting (DAP), growth parameters and gall formation at 6 weeks after planting, and growth parameters, gall formation, and yield and grade of harvest (10 plants/plot) at end of season. Other naturally occurring diseases such as Fusarium wilt, stem rot, white mold, bacterial wilt, and virus incidence were assessed in the field throughout the season.

Three plants were taken from each plot at approximately 45 DAP. Two grams of root were taken from each plant, consisting of a combination of crown tissue and lateral and fine roots. Tissue from each plant was surface sterilized with 70% ethanol for 20 s. Root tissue was combined with sterile water for a 1:10 dilution and macerated for 15 s using a Seward Stomacher® 80 lab blender (Seward Ltd, London, England). A 500 microliter aliquot of the root suspension was spread onto three plates each of the following selective media: PARP (Jeffers and Martin, 1986), selective for *Pythium* spp., consisting of corn meal agar (17 g L⁻¹, Difco brand, Fisher Scientific), pimaricin (10 mg/L of 50% a.i. Delvocid, Gist-Brocades Food Ingredients Inc., King of Prussia, PA), ampicillin (250 mg/L, 100% a.i., Sigma, St. Louis, MO), rifampicin (10 mg/L, Sigma), and pentachloronitrobenzene (134 mg/L of 75% a.i. Terrachlor WP, Uniroyal Chemical Company) and PARPH (PARP with hymexazol (50 mg/L 99.4% a.i., Sankyo Co, LTD, Tokyo, Japan) (Mitchell and Kannwischer-Mitchell, 1992; Tsao, 1983), selective for *Phytophthora* spp., and Komada's medium (Komada, 1975), selective for *Fusarium* spp. Root samples were diluted prior to plating on Komada's medium. PARP and PARPH plates were placed in a 28°C chamber for 24 h. Komada's medium was placed at 25°C. The number of *Pythium* spp. and *Phytophthora* spp. colonies formed from the 0.1 g sample were then counted after 24 h. Identification of genera was confirmed microscopically. Fifty *Pythium* isolates were randomly chosen for species identification and stored on sterilized hemp seed until used. These isolates were then grown out on PARP and transferred to plates containing diluted pond water and sterilized grass blades to induce formation of sporangia.

Damage from root-knot nematodes was assessed by counting the total number of galls or by using a root gall index based on a scale of 1–10, with one representing 0% root galling of an individual root system and

10 representing 100% root galling (Zeck, 1971). Overall root health was assessed using a subjective root rating utilizing a 1–5 scale for root condition where 1=good condition and 5=poor condition. Tomato and pepper plots were harvested two or three times during the season. Mature green or red tomato fruit were picked, culled and graded for size (small–extra large) using a standard grading line. Peppers were harvested and hand graded (small–jumbo) according to industry standard sizing.

Statistical analysis

Data were statistically analyzed according to standard procedures including SAS general linear model (GLM), least significant difference (LSD), and Tukey's HSD procedures (SAS, 1998). Unless otherwise stated, all differences referred to in the text were significant at the 5% level of probability.

Results

Effects PGPR treatments on transplant growth

In both tomato and pepper, most PGPR formulations significantly increased plant growth for almost all parameters measured during both years of the study. Tomato transplant growth was increased by all PGPR treatments, but overall growth was enhanced to the greatest extent by LS254 and LS256 (Table 1). In tomato, LS254 produced a 395% increase in dry root weight and LS256 produced a 337% increase in dry root weight compared to the untreated control. Pepper transplant vigor was also increased by all PGPR treatments, but the most dramatic effect was observed with LS261, which resulted in a 565% increase in dry root weight compared to the untreated control (Table 2). In 1999, all PGPR treatments increased fresh root weight, stem diameter, and dry shoot weight in tomato compared to the untreated control, with LS256 additionally increasing fresh shoot weight and dry root weight compared to the control (Table 3). In pepper, all PGPR treatments increased fresh root and shoot weights, stem diameter, dry root, and dry shoot weights when compared to the untreated control (Table 4). Only LS256 increased stem length. In addition to highly significant growth promotion in tomato and pepper transplants in both 1998 and 1999, transplant vigor and survival in the field improved with almost all PGPR formulations. This is best illustrated

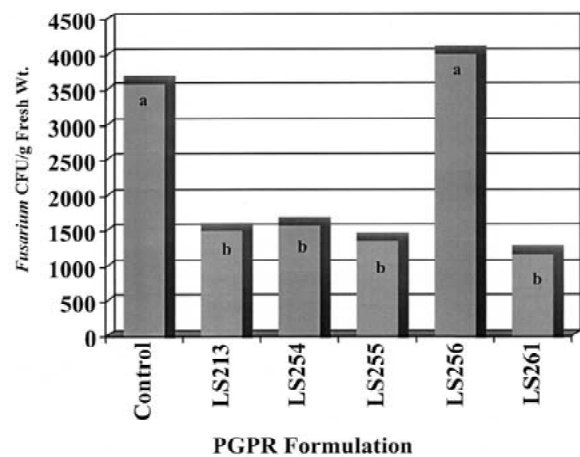


Figure 1. Effects of PGPR treatments on isolation of *Fusarium* on Komada's medium from tomato roots at approximately 45 DAP in 1998. Means of treatments with the same letter were not significantly different at $P=0.05$.

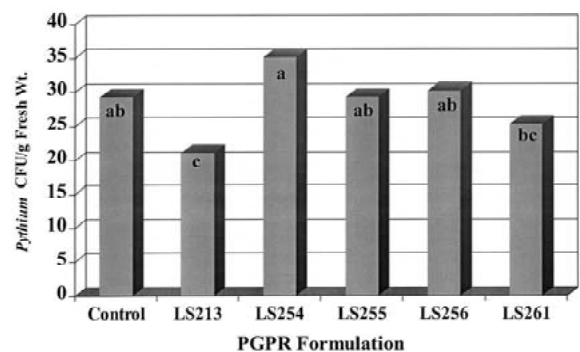


Figure 2. Effects of PGPR treatments on isolation of *Pythium* from pepper roots at approximately 45 DAP in 1998. Means of treatments with the same letter were not significantly different at $P=0.05$.

by the significant increase in tomato transplant survival at 10 DAP in 1999. Tomato transplant mortalities per plot declined from an average of more than 5.0 in the untreated control to less than 2.5 with LS213 and LS261, and to less than 1.5 for LS256.

Effects of PGPR treatments on disease

Visual field evaluation ratings for naturally occurring diseases on tomato did not indicate any effect of PGPR treatments on incidence of *Fusarium* wilt, early blight (caused by *Alternaria solani*), or tomato yellow leaf curl virus. Evaluation of foliar diseases in pepper revealed no differences relative to soil or transplant treatments. However, isolation of *Fusarium* spp. from tomato roots at 45 DAP in 1998, resulted in significantly lower colony counts for all PGPR

Table 1. Effects of PGPR treatments on 'Solar Set' tomato transplants 1998

Treatment	Stem length (cm)	Stem diameter (mM)	Leaf area (cm ²)	Dry stem weight (g)	Dry root weight (g)	Dry shoot weight (g)	Dry stem weight (g)	Leaf Ratio	True leaf (no)
LS213	8.3 ^a	3.52 ^a	29.40 ^a	0.093 ^a	0.080 ^a	0.066 ^a	0.174 ^a	1.19 ^a	3.7 ^a
LS254	10.7 ^a	4.06 ^a	35.82 ^a	0.126 ^a	0.114 ^a	0.085 ^a	0.241 ^a	1.11 ^a	4.2 ^a
LS255	10.5 ^a	3.59 ^a	37.80 ^a	0.098 ^a	0.085 ^a	0.062 ^a	0.183 ^a	1.21 ^a	4.2 ^a
LS256	12.1 ^a	3.81 ^a	43.90 ^a	0.122 ^a	0.112 ^a	0.073 ^a	0.234 ^a	1.10 ^a	4.2 ^a
LS261	10.4 ^a	3.76 ^a	39.08 ^a	0.104 ^a	0.091 ^a	0.069 ^a	0.196 ^a	1.16 ^a	4.0 ^a
Control	5.4	2.28	8.38	0.038	0.025	0.021	0.063	1.54	2.7
LSD (0.05)	2.1	0.34	9.67	0.018	0.021	0.013	0.037	0.17	0.6

^aIndicates values are significantly different from the untreated control.

Table 2. Effects of PGPR treatments on 'Jupiter' pepper transplants 1998

Treatment	Stem length (cm)	Stem diameter (mM)	Leaf area (cm ²)	Dry leaf weight (g)	Dry stem weight (g)	Dry root weight (g)	Dry shoot weight (g)	Leaf Ratio	True leaf (no)
LS213	4.3 ^a	1.75 ^a	16.46 ^a	0.042 ^a	0.016 ^a	0.028 ^a	0.059 ^a	2.67 ^a	4.0 ^a
LS254	4.1 ^a	1.73 ^a	11.41 ^a	0.035 ^a	0.015 ^a	0.024 ^a	0.050 ^a	2.22 ^a	4.2 ^a
LS255	4.2 ^a	1.72 ^a	13.27 ^a	0.041 ^a	0.016 ^a	0.026 ^a	0.057 ^a	2.60 ^a	4.5 ^a
LS256	4.2 ^a	1.65 ^a	11.65 ^a	0.032 ^a	0.015 ^a	0.029 ^a	0.047 ^a	2.15	4.2 ^a
LS261	5.5 ^a	1.98 ^a	23.87 ^a	0.053 ^a	0.024 ^a	0.034 ^a	0.077 ^a	2.26 ^a	5.2 ^a
Control	2.6	1.32	2.33	0.008	0.005	0.006	0.013	1.68	2.0
LSD (0.05)	0.6	0.13	4.15	0.010	0.004	0.006	0.013	0.49	0.5

^aIndicates values are significantly different from the untreated control.

Table 3. Effects of PGPR treatments on 'Solar Set' tomato transplants 1999

Treatment	Fresh root weight (g)	Fresh shoot weight (g)	Stem diameter (mM)	Shoot length (mM)	Dry root weight (g)	Dry shoot weight (g)
LS213	0.55 ^a	1.75	3.35 ^a	108.9	0.105 ^a	0.256 ^a
LS256	0.54 ^a	1.35 ^a	3.35 ^a	90.4	0.118 ^a	0.281 ^a
LS261	0.57 ^a	1.70	3.53 ^a	114.4	0.040	0.184 ^a
Control	0.25	1.75	2.68	72.2	0.027	0.080
Tukey's HSD (0.05)	0.14	0.34	0.48	45.3	0.018	0.067

^aIndicates values are significantly different from the untreated control.

Table 4. Effects of PGPR treatments on 'Jupiter' pepper transplants 1999

Treatment	Fresh root weight (g)	Fresh shoot weight (g)	Stem diameter (mM)	Shoot length (mM)	Dry root weight (g)	Dry shoot weight (g)
LS213	2.20	2.47 ^a	2.27 ^a	82.50	0.105 ^a	0.256 ^a
LS256	2.65 ^a	2.15 ^a	2.29 ^a	94.50 ^a	0.105 ^a	0.255 ^a
LS261	1.69 ^a	2.00 ^a	2.36 ^a	86.50	0.096 ^a	0.232 ^a
Control	1.39	1.22	1.62	61.00	0.065	0.126
Tukey's HSD (0.05)	0.61	0.35	0.31	26.97	0.020	0.058

^aIndicates values are significantly different from the untreated control.

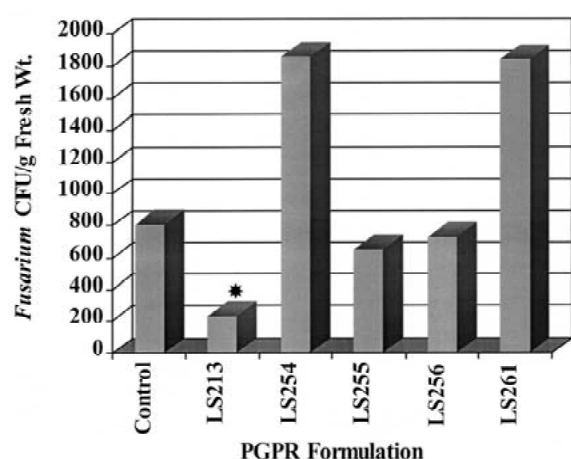


Figure 3. Effects of PGPR treatments on isolation of *Fusarium* propagules from pepper roots at approximately 45 DAP in 1998. Only treatment LS213 was significantly different from the untreated control at $P=0.05$.

treatments, except LS256, which was not different from the untreated control (Figure 1). Isolation of *Pythium* and *Phytophthora* revealed no significant differences among PGPR treatments in the numbers of colony forming units isolated from tomato roots in either year. No significant differences were found in the number of *Phytophthora* isolates from pepper roots in either year due to extremely low incidence of this pathogen. LS213 significantly decreased the number of *Pythium* CFU isolated from pepper roots at 45 DAP in 1998 compared to the untreated control (Figure 2). There were no differences among PGPR treatments in isolation of *Pythium* spp. from either crop in 1999. LS213 also had a significant effect on the number of *Fusarium* isolates from pepper roots at 45 DAP compared to the untreated control and several other PGPR treatments (Figure 3). Randomly selected isolates for

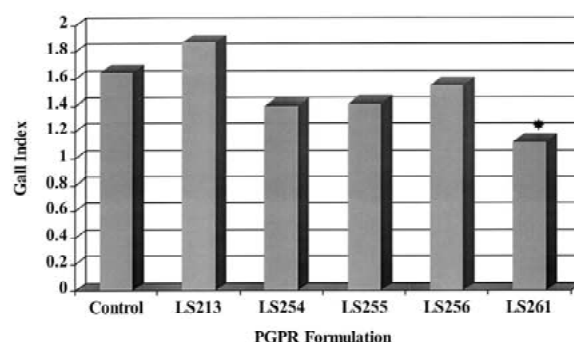


Figure 4. Effects of PGPR treatments on galling of pepper caused by root-knot nematodes (*Meloidogyne incognita*) at the end of the season in 1998. Only PGPR formulation LS261 had significantly fewer galls than the untreated control at $P=0.05$. Gall ratings were performed according to Zeck's gall index on a scale of 1–10 with 1=0% galling and 10=100% galled roots (Zeck, 1971).

species identification were found to be primarily *Pythium aphanidermatum*, although there were a small number that did not produce sporangia and were not identified.

The number of galls on tomato roots caused by root-knot nematode was not reduced and root condition was not improved by any PGPR treatment. PGPR formulation LS261 reduced nematode galling on pepper compared to the untreated control at the end of the season (Figure 4). Root condition of pepper at the end of the season, which is an overall evaluation of root health, was improved by LS213, LS256 and LS261 compared to the untreated control (Figure 5).

Effects of soil treatments on pathogen populations and disease

There were no differences in tomato transplant survival among soil treatments in 1999 when mortalities for untreated, MeBr fumigated and solarized plots

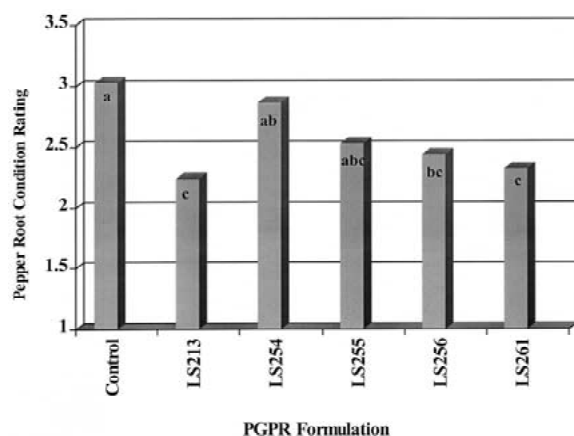


Figure 5. The effects of PGPR treatments on root condition of pepper at the end of the season in 1998. Root condition ratings were performed on a scale of 1–5 with 1=good root condition and 5=poor root condition. Means of treatments with the same letter were not significantly different at $P=0.05$.

all had between two and three mortalities/plot. However, pepper transplant mortality in solarized plots was significantly higher (4.6 mortalities/plot) than the untreated control plots (1.3 mortalities/plot).

The incidence of foliar diseases on either tomato or pepper was not affected by soil treatments. MeBr was the only soil treatment that significantly reduced galling of tomato caused by root-knot nematodes and improved root condition at the end of the season (data not shown). There were no differences in incidence of galling or root condition of tomato between solarization and untreated plots. *Pythium* colony counts from pepper roots were significantly lower in MeBr treated soil compared to untreated soil, but there was no difference between soil solarization and either the untreated control or methyl bromide (data not shown). There were no interactions between soil treatments and PGPR treatments with respect to disease incidence or isolation of pathogens from roots.

Effects of treatments on yield

During 1998, LS256 produced the highest overall yield in both tomato and pepper (Tables 5 and 6). With regards to tomato, these differences were not statistically significant. In pepper, LS256 had significantly higher yield than LS261 and LS213 in combination with soil solarization. Although tomato yields were not significantly affected by PGPR treatment when analyzed by individual soil treatment, when analyzed without regard to soil treatments, LS256 had signific-

Table 5. Tomato Total Yield 1998 (kg/plot)

	Solarization	Untreated	MeBr
Control	10.68	11.36	18.99*
LS 213	12.00	11.26	17.41
LS 254	9.89	12.46	17.44
LS 255	9.82	10.73	15.97**
LS 256	11.15	13.52	19.89
LS 261	12.65	9.39	17.70
LSD (0.05)	NS	NS	NS

*Indicates significant difference between methyl bromide treatment and the untreated control and solarization at $P=0.05$.

**Indicates significant difference between methyl bromide treatment and solarization at $P=0.05$.

Table 6. Pepper Total Yield 1998 (kg/plot)

	Solarization	Untreated	Methyl bromide
Control	24.60 ab ^a	17.56 a	26.05 a
LS 213	19.21 b	16.66 a	27.57 a*
LS 254	23.34 ab	14.85 a	20.39 a
LS 255	25.00 ab	14.50 a	22.98 a
LS256	27.96 a*	14.96 a	29.21 a*
LS 261	18.27 b	17.14 a	21.34 a
LSD (0.05)	7.76	6.11	9.048

^aLetters indicate significant differences among PGPR treatments for each soil treatment at $P=0.05$.

*Indicates significant difference between soil treatment and the untreated soil control at $P=0.05$.

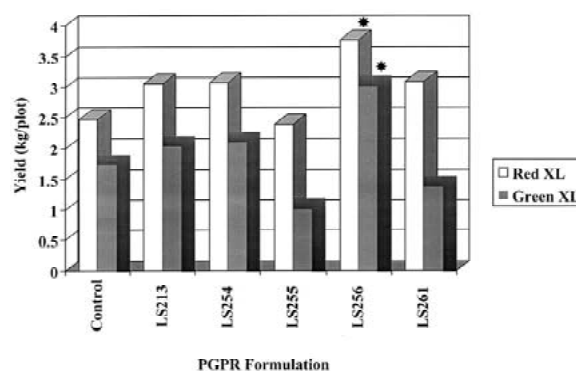


Figure 6. Effects of PGPR treatments on yield of extra large red and mature green tomatoes in 1998. Asterisks indicate means were significantly different from the untreated control at $P \geq 0.05$.

antly higher yields than LS255 for both red and mature green extra large fruit (Figure 6).

During both years of the study, MeBr significantly increased tomato yield compared to the untreated control and soil solarization (data not shown). Solarization did not increase tomato yields over the untreated control, but did increase pepper yields compared to the untreated control. In addition, pepper yields with solarization were not significantly different from yields in MeBr treated plots. There were no statistically significant interactions between transplant treatments and soil treatments, however LS256 on pepper performed best in combination with solarization.

Discussion

Results of previous experiments testing the effects of formulation components and PGPR individually have shown chitin to have some nutritional and growth promotion effects on tomato (Martinez-Ochoa et al., 2001). In those studies, the inclusion of several gram-positive PGPR in the formulation produced an additional yield response not seen in plants treated with chitin alone. In mode-of-action studies of PGPR and chitin combinations Benhamou et al. (1998) found that the combinations of chitosan and the endophytic sp. PGPR *Bacillus pumilus* induced physiological and biochemical changes in host cells at sites where the fungal pathogen *Fusarium oxysporum* attempted penetration. The aim of the current research was to further the development and refinement of potential commercial PGPR formulations. Because the organic chitin component and one of the bacterial components, *B. subtilis* strain GBO3, remained constant in all treatments, we can conclude that differences in plant growth promotion and pathogen suppression among treatments can be attributed primarily to the interactive effects of the second bacterial component in each treatment.

In addition to induction of resistance, microbial interactions in the rhizosphere may account for reductions in soilborne fungal pathogens. Previously, Nemec (1997) found that isolates of *Bacillus* survived better in planting mixes than several other potential biocontrol agents, including species of *Pseudomonas* and *Serratia*. In addition, Nemec et al. (1996) showed that *Trichoderma harzianum* delivered in the transplant plug system effectively controlled *Fusarium* crown rot, and that addition of *Bacillus subtilis* to the transplant medium provided effective control of

Phytophthora in the field. Studies on the population dynamics of our applied PGPR isolates are currently underway to determine the extent of these interactions.

Transplant mixes amended with PGPR formulations greatly enhanced transplant growth. Although both tomato and pepper transplant height was dramatically increased by most PGPR treatments, tall transplants can be undesirable. Therefore, better indicators of quality and vigor in transplants are the evaluation of stem caliper and dry root weight. PGPR treated tomato and pepper transplants showed significant increases in stem caliper and root growth. It has been shown that plants with larger root systems and stem diameters are less susceptible to environmental stresses after transplanting (Vavrina, 1996). The extent to which PGPR formulations enhanced transplant growth virtually eliminated the need for supplemental fertilizer applications during greenhouse transplant production. Reduction in fertilizer usage and increased growth rate of plants could translate into significant savings for production houses and decreased ground water pollution resulting from fertilizer run-off (Vavrina et al., 1998).

Control of pathogens after transplanting into the field differed among PGPR isolates and between crops in these studies. Previous work with LS213 on muskmelon and watermelon, resulted in increased plant growth in the greenhouse, disease suppression in the field and slightly improved yields (Vavrina, 1999). Ratings performed during the current studies to assess the incidence of naturally occurring foliar pathogens led to the conclusion that there was no systemic affect of PGPR treatments on incidence of various foliar pathogens. These results differ from those of Raupach et al. (1996), where various PGPR were reported to act as inducers of systemic resistance towards cucumber mosaic virus in tomato and to protect cucumber against the fungal pathogens *Colletotrichum orbiculare* and *Fusarium oxysporum* and the bacterial pathogen *Pseudomonas syringae* pv. *lachrymans*. However, results for soilborne pathogens were positive, in that several PGPR formulations reduced the incidence of *Pythium* isolated from pepper and of *Fusarium* isolated from both pepper and tomato. Results relating to the isolation of *Pythium* spp. are somewhat complicated due to the inability to identify all isolates as pathogenic. Due to its low incidence during both years of this study, it could not be determined if *Phytophthora* root colonization of pepper was affected by transplant treatment. Additional experimentation, in the presence of an established population of *P. capsici*,

is necessary and warranted due to the high incidence and destructive capability of this pathogen in Florida.

Although tomato did not exhibit a reduction in disease caused by root-knot nematode with PGPR treatments, pepper (which is less susceptible than tomato to root-knot nematode) did show a reduction in galling. The experimental farm in Sanford, Florida had an extremely high population of *M. incognita* and it is possible that effects of PGPR treatments on root-knot nematode incidence on tomato were masked by the aggressive nature of the population and high initial population numbers.

Soil solarization did not control the aggressive population of root-knot nematode on tomato. However, solarization did provide some protection of pepper from *Pythium*. In addition, when combined with some PGPR formulations on pepper, solarization produced yields comparable to untreated transplants grown in soil fumigated with methyl bromide. The practicality of soil solarization alone for pathogen control is questionable when one considers that in Florida, many vegetable producers rely on profits from production of a spring crop following either tomato or pepper the previous fall. The bed infrastructure, including plastic and drip irrigation tubing are left in place and reutilized. In the past, methyl bromide has provided sufficient pathogen control to allow for production of the spring crop. Solarization has potential to be effective for fall production under low levels of pathogen pressure, but would most likely become ineffective during the second crop production period unless additional pest control measures were employed.

Alternative fumigants are considered the most promising short-term replacement for MeBr. However, some of these chemicals may have limited life spans due to toxicological and environmental problems, leaving producers vulnerable to future regulatory policies. Still, microbial biocontrol agents alone will not replace MeBr. The development of production systems not dependent on the use of MeBr will require an integrated approach utilizing a variety of alternative technologies to maximize yield and maintain pest damage below an economic threshold. The use of one or more PGPR treatments in management systems including alternative chemical fumigants, or solarization may allow acceptable levels of crop production. The addition of microbial agents to transplant mixes is an ideal delivery system for biocontrol agents on transplanted vegetable crops. This technology is also compatible with organic production systems. The

greatest benefits of PGPR transplant amendments include the reduction of application rates for fertilizers and growing time during greenhouse production, increased transplant survival in the field, reduced incidence of some pathogens, and enhanced yield in some crops.

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 BioYieldTM (Gustafson LLC, Plano, TX). We wish to acknowledge Donald S. Kenney of Gustafson LLC, Plano, TX for technical assistance, Uniroyal Chemical Company, Inc. for use of their research farm, Bryan Beaty for assistance with all aspects of this project, Dan Chellemi and Ed Killer for assistance with soil solarization, and Amanda Rinehart for assistance with manuscript preparation.

References

- Anonymous 1997 Florida Agricultural Statistics. FL Ag. Statistics Service, Orlando, FL.
- Benhamou N and Thériault G 1992 Treatment with chitosan enhances resistance of tomato plants to the crown and root rot pathogen *Fusarium oxysporum* f. sp. *radicis lycopersici*. *Physiol. Mol. Plant Pathology* 41, 33–52.
- Benhamou, N, Lafontaine P J, Nicole M 1994 Induction of systemic resistance to *Fusarium* crown and root-rot in tomato plants by seed treatment with chitosan. *Phytopathology* 84, 1432–1444.
- Benhamou, N, Kloepper J W, Tuzun S 1998 Induction of resistance against *Fusarium* wilt of tomato by combination of chitosan with an endophytic bacterial strain: ultrastructure and cytochemistry of the host response. *Planta* 204, 153–168.
- Chellemi D O, Olson S M, Mitchell D J, Secker, I, McSorley, R 1997 Adaption of soil solarization to the integrated management of soilborne pests of tomato under humid conditions. *Phytopathology* 87, 250–258.
- Chellemi D O, Olson S M, Scott J W, Mitchell D J, McSorley, R 1993 Reduction of phytoparasitic nematodes on tomato by soil solarization and genotype. *Supplement to Journal of Nematology* 25, 800–805.
- Cook J R and Baker K F 1983 The Nature and Practice of Biological Control of Plant Pathogens. APS Press, St. Paul MN, 539 pp.
- Gilreath J P, Noling J W, Locascio S J, Chellemi D O 1999 Effect of MeBr, 1,3-dichloropropene + chloropicrin with pebulate and soil solarization on soilborne pest control in tomato followed by double-cropped cucumber. *Proc. Fla. State Hort. Soc.* 112, 292–297.
- Hoitink H A J and Fahy P C 1986 Basis for the control of soilborne plant pathogens with composts. *Annu. Rev. Phytopathol.* 24, 93–114.

- Jeffers S N and Martin S B 1986 Comparison of two media selective for *Phytophthora* and *Pythium* spp. Plant Disease 70, 1038–1043.
- Kloepper J W, Leong J, Teintze M and Schroth M N 1980 Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature 286, 885–886.
- Kokalis-Burelle N, Rodríguez-Kábana R, Weaver C F and King P S 1994 Evaluation of powdered pine bark for control of *Meloidogyne arenaria* and *Heterodera glycines* on soybean. Plant and Soil 162, 163–168.
- Komada H 1975 Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Protection Research 8, 114–125.
- Martínez-Ochoa N, Kokalis-Burelle N, Kloepper J W and Rodríguez-Kábana R 2001 Rhizobacteria, organic amendments, and botanical aromatics for the management of *Meloidogyne* spp. in tomato transplants. Plant Disease 85: In Press.
- Mitchell D J and Kannwischer-Mitchell M E 1992 Methods for Research on Soilborne Phytopathogenic Fungi. Pages 31–38, L L Singleton, J D Mihail and C M Rush, eds. APS Press, St. Paul, MN 265 pp.
- Nemec S 1997 Longevity of microbial biocontrol agents in a planting mix amended with *Glomus intraradices*. Biocontrol Science and Technology 7, 183–192.
- Nemec S, Datnoff L E and Strandberg J 1996 Efficacy of biocontrol agents in planting mixes to colonize plant roots and control root diseases of vegetables and citrus. Crop Protection 15 (8), 735–742.
- Raupach G S, Liu L, Murphy J F, Tuzun S, Kloepper J W 1996 Induced systemic resistance in cucumber and tomato against cucumber mosaic cucumovirus using plant growth-promoting rhizobacteria (PGPR). Plant Dis. 80, 891–894.
- SAS Institute, Inc. 1998 Version 7.01, Cary, NC: SAS Institute, Inc.
- Tsao P H 1983 Factors affecting isolation and quantification of *Phytophthora* from soil. Pages 219–236 In: *Phytophthora: Its Biology, Taxonomy, Ecology, and Pathology*. APS Press, St Paul, MN.
- Vavrina C S 1996 An introduction to the production of containerized vegetable transplants. Univ. FL., Cooperative Extension Service, Bulletin No. 302.
- Vavrina C S, Hochmuth G J, Cornell J A, Olson S M 1998 Nitrogen fertilization of Florida-grown tomato transplants: seasonal variation in greenhouse and field performance. HortScience 33, 251–254.
- Vavrina C S 1999 The effects of LS213 (*Bacillus pumilus*) on plant growth promotion and systemic acquired resistance in muskmelon and watermelon transplants and subsequent field performance. Proc. Int. Symp. Stand Establishment 107–111.
- Weller D M 1988 Biological control of soilborne pathogens in the rhizosphere with bacteria. Ann. Rev. Phytopathol. 26, 379–407.
- Zeck W M 1971 A rating scheme for field evaluation of root-knot nematode infestation. Pflanzenschutz-Nachr. 24, 141–144.
- Zehnder G W, Murphy J F, Sikora E J, Kloepper J W 2001 Applications of rhizobacteria for induced resistance. Europ. J. Plant Pathol. 107 (1), 39–50.

Section editor: T.C. Paulitz