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Plant growth promoting rhizobacteria via a transplant plug delivery system in the production of drip irrigated pepper.

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Introduction

In 1997-98, over 20,000 acres of peppers were grown in Florida. This acreage has varied over the past several years as competition from Mexico has stiffened, *Phytophthora capsici* has emerged as a new threat and returns on investments have diminished. To stay competitive and comply with regulations, FL growers must become more efficient, increase yields and reduce their dependency on pesticides in keeping with consumer desires and environmentally driven legislation. One way to perhaps satisfy all of the above conditions may be found in the technology of plant growth promoting rhizobacteria and systemic acquired resistance.

Kodiak is a biological seed/hopperbox treatment marketed by Gustafson Inc (Plano, TX) for use in agronomic crops. Kodiak contains *Bacillus subtilis* (GB03) which has been shown to promote plant growth and increase yield in cotton. Previous work with *B. subtilis* in conjunction with *B. amyloliquefaciens* (LS213) in cantaloupe and watermelon (SWFREC Station Report - VEG 98.7) have been shown to increase plant growth in the greenhouse, extend some disease prevention in the field and have a relatively minor effect on yield (reduced average fruit weight at first harvest). The results of the cantaloupe and watermelon work were compelling enough to foster a second look at this product in 1998 – 1999.

The objective of this study was to apply several *Bacillus* treatments known to have biologic activity in accordance with a Gustafson protocol on drip irrigated pepper to test the hypotheses of enhanced crop growth, disease prevention and yield.

Methods

A trial, established at the Southwest FL Research and Education Center (SWFREC) of the University of Florida in Immokalee, FL, included 10 treatments, nine *Bacillus* treatments and the control (Table 1.) All *Bacillus* treatments were added to a soilless medium (70% peat, 30% vermiculite) prior to seeding and transplant production. `Aladdin' (Peto Seed, Saticoy, CA), a yellow bell at maturity, was used as the pepper cultivar. The pepper transplants were grown in accordance with south FL standards at a commercial production facility (TransGro, Immokalee, FL). Treatments were randomized and replicated (4) within the production facility by SWFREC personnel. Upon completion of the transplant cycle (6 weeks), five plants per replication per treatment were sampled to include: root/stem/leaf dry weight (DW), stem length, stem diameter, chlorophyll rating (via SPAD meter), leaf area and number of true leaves.

Table 1. *Bacillus* treatments used in the production of pepper transplants.

Treatment	PGPR ID*
LS213	*B. amyloliquefaciens*
LS254	*B. pumilus***
LS255	*B subtilis*
LS256	*B. pumilus*
LS257	*B. pumilus*
LS258	*Brevibacillus brevis*
LS259	*Bacillus subtilis*
LS260	*Paenibacillus macerans*
LS261	*Bacillus cereus*
Control	

*All contain B. subtilis strain GB03 plus the 2nd indicated strain
**Different strains of B. pumilus are from different origins

All transplant treatments were sampled, however only treatments LS213, 254, 255 and 256 were taken to the field for subsequent testing due to space limitations. The remaining pepper transplants were set in the SWFREC sustainable agriculture field on 1 Oct., 1998. Once set in the field an additional treatment was employed. Five applications of Actigard (Novartis) were applied (3.5 g. per 100 liters water) at weekly intervals beginning 21 DAP. Actigard is purported to induce systemic resistance via activation of the salicylic acid pathway.

The sustainable field featured plots with and without compost amendments and either solarized, methyl bromide fumigated or unfumigated soils with drip irrigation. Solarization began July 23, 1998 and continued for 60 days, using clear high-density 0.75-mil polyethylene containing UV light inhibitors (Sonoco Products Co., Orlando, FL). Biosolids were applied prior to bed formation at the rate of 37 $Mg.ha^{-1}$ for each compost plot. Methyl bromide and chloropicrin (98:2) were applied on Sept 10, 1998. Methyl bromide was applied at the rate of 336 $kg.ha^{-1}$. Drip tubing (0.67 GPM/30m) was positioned in the center of the bed during plastic mulch application. The pepper was planted on 10" X 10" spacing in double rows, on raised beds 0.81 m wide, 0.1 m high, and 1.8 m between centers and 15 m long. Beds were covered with white-on-black polyethylene mulch. Solarization mulch was removed and replaced with white-on-black polyethylene mulch.

All fertilizer was injected via drip at 428N-0P-178K $kg{\cdot}ha^{-1}$ in the non-biosolid plots and at 2141N-0P-90K $kg{\cdot}ha^{-1}$ in the biosolids plots. No granular fertilizer was applied at planting. Nitrogen was reduce by 50% in the biosolids plots to compensate for N mineralized from the biosolid amendment. The plants were monitored for insects and diseases and pesticide were applied as needed according to Univ. of Florida Extension guidelines.

Stand establishment data were taken 18, 39, 53 DAP to determine plant growth and fruit development in the field by removing the aerial portion of two plants per treatment at each sample period. Disease incidence and severity was assessed 66, 80 and 98 DAP. Peppers were harvested 71, 83 and 100 DAP and separated by weight, number into fancy (extra large), number one (large), and number two (medium) grade fruit according to USDA specifications. The study was set out with four replications as a split, split plot design, where + or - compost represented the main plots and the soil sterilization technique represented the subplots.

Results

In general no *Bacillus* by land preparation (compost vs. noncompost) or *Bacillus* by bed preparation (solarized vs. fumigated vs. unfumigated) interactions occurred during this study. Therefore, only the main effect of the biologicals will be discussed in the following sections. Where interaction did occur, a full explanation will be undertaken. Additionally, while the $p \leq 0.10$ was calculated the results of this analysis will not be shown in the tables if it was not significant.

Transplant Growth

The *Bacillus* treatments were coated on a carrier of flaked chitin for ease of application. Though chitin itself is known to have plant growth promotion capabilities a separate chitin control was not used. The treatments were therefore viewed as individual formulations.

All *Bacillus* treatments produced larger plant shoots than the untreated control (Table 2) for the parameters measured (total shoot DW, leaf DW, stem DW, leaf area, stem length, stem diameter, number of leaves, chlorophyll rating) with only minor exceptions (LS259 – stem diameter, LS259, 256, 254 – leaf no.). The leaf:stem ratio, an indicator of stem to leaf partitioning, was similar across treatments ($p \leq 0.05$) indicating that *Bacillus* treatment did not affect partitioning. The LS257 leaf to stem ratio reflected a slight increase in leaf development (i.e., > leaf area) at the expense of stem development ($p \leq 0.10$), but whether this has a practical significance is doubtful.

Control root DW was significantly different from *Bacillus* treatments LS213, 254, 255, and 259 at $p \leq 0.10$. All *Bacillus* treatments reduced the root to shoot ratio (partitioning). Essentially, each unit of *Bacillus* treated roots supports more shoot tissue (2.63 units) than the untreated control (2.16 units).

In-Field Plant Development

Plant growth enhancement by *Bacillus* treatment did not readily translate to improved growth in the field during the establishment phase (Table 3). Plant samples taken 18 and 53 DAP showed no difference in dry matter accumulation attributable to treatment. However, the plant samples of 39 DAP, showed the LS254 treatment to have greater shoot DW than both the control and the Actigard treatment. The LS255 treatment also exhibited greater shoot DW than the Actigard treatment, 39 DAP.

Fruit development 39 DAP was retarded by Actigard when compared to the *Bacillus* treatments (Table 3.) Fruit development seemed to lag for both the control and Actigard treatment 53 DAP as well (Table 3.) While the control generally produced the same total fruit number as the *Bacillus* treatments, the total fruit weight for the LS254, 255 and 256 treatments was greater. The increase in average fruit weight of treatments LS254 and 255 confirmed the weight gain hypothesis. Actigard treatment generally reduced pepper fruit number (large, total) and weight (small, large, total) 53 DAP.

Disease Rating

Bacterial spot (Xanthamonas campestris) infested the pepper just before first harvest. Incidence (% plants infected) and severity (% leaves infected) of the infestation was assessed 66, 80 and 98 DAP (Table 4). Sixty-six DAP the LS213 and 254 treatments had spot incidence equal to the foliar applied Actigard ($p \leq 0.01$) while LS255, 256 and the control showed significantly greater incidence. Two weeks later (80 DAP), LS213 continued to show reduced spot incidence compared to Actigard, and both LS213 and 254 exhibited severity comparable to Actigard as well. Almost 3 weeks later (98 DAP), LS213 and 256 showed spot incidence comparable to Actigard while severity was comparable for all treatments except the control and LS254. Significantly reduced incidence (66 DAP- LS254) or severity (98 DAP- LS213) was also noted when the *Bacillus* treatments were compared to the control.

Interestingly, the 80 DAP disease rating revealed an interaction effect between treatments and the presence or absence of compost:

Compost spot incidence:	Control>	LS254>	LS256>	LS213>	LS255>	Actigard
	(39)	(33)	(31)	(31)	(29)	(16)

Noncompost incidence:	Control>	LS255>	LS254>	Actigard>	LS256>	LS213
	(15)	(13)	(13)	(12)	(11)	(8)

Significantly more disease occurred in the compost plots, presumably due to the higher nitrogen levels provided by mineralization of the compost.

Pepper Yield

LS256 produced more extra-large fruit (number and weight) than the control at first harvest (Table 5). This trend was not pronounced for large or medium fruit nor did it effect total first harvest fruit yield. Actigard was clearly not beneficial for pepper fruit development and will not be discussed further.

Second harvest is often considered the compensation harvest, i.e., the harvest where low yielding treatments generally produce higher yields. Second harvest extra-large fruit production depicts this phenomenon (though not dramatically) where LS256 produced the fewest fruit and all other treatments yielded more fruit (Table 6). LS254 produced more large (weight and number) and medium (number only) fruit than the control leading to more total fruit (number) at second harvest. This compensation surge by LS254 at second harvest resulted in a greater number of total fruit when compared to LS256 and a greater total weight of fruit when compared to LS255.

Clearly, the third harvest was the compensation harvest for Actigard (Table 7). While *Bacillus* and the control treatments yielded similarly at third harvest the control produced heavier individual fruit (average fruit weight) than LS213. Combining all three harvests revealed that LS254 produced more total fruit (number) than the control and LS213 and 255 (Table 87). The increase posted by LS254 appeared to be the result of greater large fruit production (number and weight) when compared to the control.

Discussion

Transplant growth enhancement via the formulation of *Bacillus* treatments plus chitin seen here was predominantly a shoot phenomenon. All *Bacillus* treatments reduced the root to shoot ratio (partitioning). This may have negative ramifications if water or nutrients become limiting.

While plant growth enhancement by *Bacillus* treatment was not consistent throughout the establishment phase the LS254 treatment had a greater shoot DW than the control 39 DAP. Fourteen days later the control produced the same total number of pre-harvest fruit as the *Bacillus* treatments, but the total fruit weight for the LS254, 255 and 256 treatments was greater. This would suggest that as the control continued to develop leaf and stem tissue the *Bacillus* treated pepper shifted emphasis to sizing fruit.

The fact that LS213 and 254 treatments were able to induce resistance to bacterial spot and thereby reduce incidence and severity to a level comparable to Actigard was quite encouraging. LS256 figured in this phenomenon as well. Reduced incidence (66 DAP- LS254) or severity (98 DAP- LS213) was also noted with *Bacillus* treatments vs. the control. These data indicate that while bacterial spot may occur on *Bacillus* treated pepper, the reduction in incidence and severity conveyed by the treatments will help the grower in control of the infestation. The shift in the hierarchy of induced resistance levels by the *Bacillus* treatments on composted vs. noncomposted land may reflect the need to target certain *Bacillus* treatments under specific cultural conditions.

LS256 produced more extra-large fruit (number and weight) than the control at first harvest. Generally, if an advantage exists for a treatment in either the transplant or early establishment phase that advantage will manifest in two ways; more fruit (number and/or weight) or larger fruit at first harvest. The compensation surge by LS254 at second and third harvest resulted in more total fruit (number) than the control and LS213 and 255 treatments. The only negative to *Bacillus* yield data may be the fact that the control produced heavier individual fruit (average fruit weight) than LS213. This may be a disadvantage during packing and shipping, as thinner walled fruit tend to bruise and crack more easily.

The benefits of these *Bacillus* treatments were evident in four aspects of pepper production; transplant growth, stand establishment, yield and disease prevention. The thought of incorporating something in the transplant soilless mix that conveys multiple enhancements is encouraging, therefore, the results of this trial are quite positive.

Table 2. Pepper transplant sample data at six weeks after seeding.

Treatment	Stem Length (cm)	Stem Diameter (mm)	Chlorophyll Rating	Leaf Area (cm^2)	True Leaf (no.)	Dry Leaf (g)	Dry Stem (g)	Dry Root (g)	Dry Top (g)	Leaf Stem Ratio	Root Shoot Ratio
LS213	12.6	2.42	26.2	30.43	6.2	0.0928	0.0808	0.0565	0.1736	1.16	0.330
LS254	11.8	2.24	25.9	25.09	5.6	0.0689	0.0642	0.0523	0.1331	1.07	0.393
LS255	11.9	2.23	25.5	25.95	5.8	0.0750	0.0685	0.0562	0.1435	1.10	0.396
LS256	12.2	2.23	25.0	26.56	5.6	0.0770	0.0708	0.0587	0.1478	1.08	0.396
LS257	13.6	2.32	26.4	31.54	6.0	0.0887	0.0859	0.0596	0.1746	1.03	0.348
LS258	11.8	2.25	25.2	26.85	6.0	0.0783	0.0693	0.0602	0.1476	1.13	0.410
LS259	12.1	2.21	25.5	26.36	5.6	0.0750	0.0695	0.0533	0.1445	1.08	0.372
LS260	12.7	2.32	26.0	28.76	5.8	0.0830	0.0774	0.0626	0.1605	1.07	0.393
LS261	12.2	2.24	26.4	27.65	5.9	0.0840	0.0737	0.0611	0.1577	1.14	0.386
Control	10.0	2.10	23.1	19.73	5.2	0.0565	0.0509	0.0496	0.1074	1.11	0.463
LSD 5%	1.0	0.12	1.1	2.83	0.5	0.0100	0.0086	0.0073	0.0171	NS	0.051
LSD 10%	-	-	-	-	-	-	-	-	-	0.08	-

Table 3. The effect of biologicals on in-field pepper dry weight and fruit development (number and weight)*.

Treatment	18 DAP Shoot DW (g)	39 DAP Shoot DW (g)	39 DAP Fruit (no.)	39 DAP Fruit Wt (g)	53 DAP Shoot DW (g)	53 DAP Small Fruit (no.)	53 DAP Small Fruit (g)	53 DAP Large Fruit (no.)	53 DAP Large Fruit (g)	53 DAP Total (no.)	53 DAP Total Wt (g)	Ave. Fruit Wt (g)
LS213	0.764	8.476	1.4	1.19	17.4	2.9	24.4	2.1	93.3	5.0	117.8	24.9
LS254	0.777	9.378	1.6	1.92	17.6	2.1	17.9	2.3	120.3	4.4	138.2	32.4
LS255	0.719	9.259	1.5	1.83	18.0	2.6	25.4	2.1	117.1	4.7	142.5	30.7
LS256	0.750	9.148	1.8	1.53	17.9	2.9	23.6	2.1	112.5	5.0	136.1	27.3
Check	0.654	8.256	1.2	0.82	18.1	3.1	25.4	1.6	77.8	4.6	103.2	22.9
Actigard	0.689	8.060	0.6	0.10	16.2	1.9	16.1	0.9	42.4	2.8	58.5	20.6
LSD 5%	NS	1.048	0.6	1.27	NS	0.8	7.1	0.5	26.1	0.8	24.8	5.9

*Mean from 2 plants per plot.

Table 4. Disease incidence and severity 66, 80 and 98 DAP.

	66 DAP		80 DAP		98 DAP	
Treatment	Incidence (%)	Severity*	Incidence	Severity	Incidence	Severity
LS213	12.7	4.6	18.4	4.8	72.1	19.9
LS254	11.5	3.9	20.7	5.0	78.7	23.0
LS255	14.7	6.0	22.4	6.6	76.3	21.0
LS256	16.2	4.1	21.8	6.5	71.8	21.5
Check	20.7	6.7	26.3	7.8	78.5	27.0
Actigard	4.3	2.0	8.2	2.0	61.2	14.7
LSD5%	NS	NS	10.6	3.7	11.7	7.1
LSD10%	9.1	NS	-	-	-	-

*Severity = Percent leaf damage on diseased plants.

Table 5. First harvest pepper yield for *Bacillus* treatments and Actigard in a sustainable agriculture setting, December 1999.

Treatment	Extra-large (no.)	Extra-large (lb.)	Large (no.)	Large (lb.)	Medium (no.)	Medium (lb.)	Total (no.)	Total (lb.)	Average Fruit Wt (lb.)
LS213	19.3	8.4	22.4	7.2	1.0	0.2	42.7	15.9	0.368
LS254	18.2	8.1	22.7	7.4	0.9	0.2	41.8	15.7	0.366
LS255	18.5	8.4	23.4	7.7	0.8	0.2	42.8	16.3	0.375
LS256	21.0	9.3	22.4	7.4	1.0	0.3	44.5	16.9	0.377
Check	18.0	7.9	22.2	7.1	0.8	0.2	41.1	15.2	0.367
Actigard	10.6	4.7	10.8	3.4	0.6	0.1	22.0	8.2	0.367
LSD 5%	2.7	1.2	3.8	1.2	NS	NS	5.1	2.0	NS

Table 6. Second harvest pepper yield for *Bacillus* treatments and Actigard in a sustainable agriculture setting, January 1999.

Treatment	Extra-large (no.)	Extra-large (lb.)	Large (no.)	Large (lb.)	Medium (no.)	Medium (lb.)	Total (no.)	Total (lb.)	Average Fruit Wt (lb.)
LS213	3.8	1.8	34.8	12.2	12.1	2.8	50.7	16.8	0.332
LS254	3.5	1.6	36.2	12.8	15.2	3.4	54.9	17.8	0.325
LS255	3.6	1.7	33.3	11.8	12.0	2.7	48.9	16.2	0.330
LS256	3.3	1.5	34.5	12.4	12.2	2.8	50.1	16.8	0.336
Check	4.2	2.0	32.7	11.5	12.9	2.9	49.8	16.5	0.331
Actigard	3.2	1.4	24.5	8.5	11.1	2.4	38.8	12.3	0.319
LSD 5%	NS	NS	3.3	1.2	NS	NS	4.5	1.5	NS
LSD 10%	NS	NS	-	-	2.3	0.6	-	-	NS

Table 7. Third harvest pepper yield for *Bacillus* treatments and Actigard in a sustainable agriculture setting, January 1999.

Treatment	Extra-large (no.)	Extra-large (lb.)	Large (no.)	Large (lb.)	Medium (no.)	Medium (lb.)	Total (no.)	Total (lb.)	Average Fruit Wt (lb.)
LS213	1.8	0.8	11.2	3.7	7.1	1.5	20.1	6.0	0.293
LS254	2.2	1.0	12.9	4.4	7.9	1.7	23.0	7.2	0.304
LS255	1.4	0.7	10.6	3.6	7.8	1.7	19.8	6.0	0.305
LS256	1.6	0.8	12.5	4.3	7.2	1.6	21.2	6.6	0.306
Check	2.3	1.1	11.5	4.1	8.1	1.8	21.9	7.0	0.314
Actigard	3.8	1.9	16.8	5.8	8.7	2.0	29.3	9.7	0.323
LSD 5%	1.1	0.6	2.9	1.1	NS	NS	4.6	1.5	0.015

Table 8. Combined harvests of pepper yields for *Bacillus* treatments and Actigard in a sustainable agriculture setting, 1998-1999.

Treatment	Extra-large (no.)	Extra-large (lb.)	Large (no.)	Large (lb.)	Medium (no.)	Medium (lb.)	Total (no.)	Total (lb.)	Average Fruit Wt (lb.)
LS213	25.0	11.0	68.3	23.2	20.2	4.5	113.5	38.7	0.339
LS254	23.8	10.8	71.8	24.6	24.1	5.4	119.7	40.7	0.337
LS255	23.5	10.7	67.4	23.1	20.7	4.6	111.5	38.4	0.342
LS256	26.0	11.6	69.4	24.0	20.4	4.6	115.8	40.3	0.347
Check	24.5	11.1	66.5	22.6	21.8	4.9	112.8	38.7	0.341
Actigard	17.7	8.0	52.0	17.7	20.4	4.5	90.1	30.2	0.334
LSD 5%	3.3	1.5	4.6	1.7	NS	NS	6.3	2.4	NS
LSD10%	-	-	-	-	NS	NS	-	-	0.008