

# Effects of plant growth-promoting rhizobacteria on bell pepper production and green peach aphid infestations in New York

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## Abstract

Plant growth-promoting rhizobacteria (PGPR) are known in various cropping systems to increase plant growth and vigor, as well as induce resistance to pathogens and pests. A commercial soil amendment containing a mixture of two species of *Bacillus* PGPR (*Bacillus subtilis* and *Bacillus amyloliquefaciens*) was evaluated for impact on germination and initial growth of bell pepper plants, efficacy against the green peach aphid, *Myzus persicae* Sulzer, and yield enhancement. Studies in the greenhouse revealed that pepper germination rate and dry weight of seedlings grown with or without *Bacillus* spp. did not differ significantly. In the field, the PGPR did not significantly reduce aphid populations compared to control plants, whereas imidacloprid was highly effective. An increase in yield compared with control plants was observed in the 2003 season, but not the following two seasons. Aphid pressure was high in 2003, and plants grown in the presence of *Bacillus* spp. exhibited substantial tolerance to aphids. That is, there were significantly higher populations of the green peach aphid on both control and PGPR-treated plants compared with imidacloprid-treated plants. However, fruit yield in the *Bacillus* spp. treatment was significantly greater than yield in the control treatment and similar to yield in insecticide-treated plots. *Bacillus* PGPR could be useful in a *M. persicae* management program for pepper plants grown in locations with consistently high aphid pressure.

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## 1. Introduction

Rhizobacteria colonize plant roots and consume root exudates and lysates (Pieterse et al., 2002; Antoun and Prevost, 2006). Certain strains are referred to as plant growth-promoting rhizobacteria (PGPR), which can be used as inoculant biofertilizers (Kennedy et al., 2004). These bacteria include species of *Pseudomonas* and *Bacillus*, both of which provide direct and indirect effects on plant growth and pest resistance (Persello-Cartieaux et al., 2003; Kennedy et al., 2004; Nelson, 2004). While a positive impact of PGPR on initial growth of bell pepper, *Capsicum annuum* L., has been described previously (Kokalis-Burelle et al., 2002; Garcia et al., 2004; Joo et al., 2005; Russo, 2006), none of the previous studies were done under environmental and cultural conditions found in

the Northeastern United States. Thus, the utility of PGPR as inoculant biofertilizers in this region is not well understood.

PGPR can directly benefit plant growth by increasing nitrogen uptake, synthesis of phytohormones, solubilization of minerals, and iron chelation (Bowen and Rovira, 1999). Some PGPR may suppress soil-borne pathogens by producing siderophores, antimicrobial metabolites, or competing for nutrients and/or niches (Nelson, 2004). Indirectly, some PGPR stimulate an increase in resistance to pathogens and pests that feed on leaves by activating the formation of physical and chemical barriers in the host, a phenomenon referred to as induced systemic resistance (Persello-Cartieaux et al., 2003; Ryu et al., 2003; Pieterse et al., 2002; Kloepper et al., 2004; Bostock, 2005).

Induced resistance is a phenomenon documented in many plant–insect and plant–pathogen interactions (Zehnder et al., 1997; Zehnder et al., 2001; Conrath et al., 2006; Stout et al., 2006; Tuzun and Bent, 2006).

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The concept of activating a plant's defense pathways to control pests in agriculture is appealing, though difficult to implement effectively. There are several examples of plants treated with PGPR, or with chemical inducers of the same plant defense response pathways, which show a decrease in insect herbivory. Zehnder et al. (1997) used PGPR to reduce feeding by the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barber, six to ten-fold on cucurbits. Boughton et al. (2006) reported that plants treated with defense elicitors caused green peach aphid, *Myzus persicae* Sulzer, population growth was significantly slowed compared with control plants. Additionally, white clover and *Medicago* plants grown in the presence of a *Pseudomonas*-like PGPR were better able to resist effects of blue-green aphids, *Acyrtosiphon kondoi* Shinji (Kempster et al., 2002). Stout et al. (2002) speculated that the delay in population growth and population size of cotton aphids, *Aphis gossypii* Glover, on cucumbers was due to a *Bacillus*-containing PGPR treatment. Several *Bacillus* PGPR species applied to tomato as seed treatments were found to reduce whitefly nymph densities 40–43%, but did not consistently decrease the severity of whitefly-transmitted tomato mottle virus or increase yield (Murphy et al., 2000).

The green peach aphid, *M. persicae*, is a pest of pepper in New York, attacking over 75% of the acreage annually (Frantz et al., 2004). Large numbers of aphids can reduce plant vigor and cause defoliation. While many insecticides are registered for *M. persicae* control on pepper, there is a need for biologically based products to control infestations. A PGPR would be of great value, especially to conserve natural enemies and to avoid potential problems encountered when some insecticides fail to control populations that have developed resistance (Devonshire, 1989; Minks and Harrewijn, 1989; Wang et al., 2002; Reiners and Petzoldt, 2007).

The goal of this study was to determine the utility of a commercially available *Bacillus* PGPR product for improving plant growth and controlling *M. persicae* on field-grown peppers in New York. The hypotheses were that the *Bacillus* spp. would (1) enhance germination and initial plant growth of pepper seedlings before transplanting in the field, (2) reduce populations of *M. persicae* on pepper, and (3) contribute to greater fruit yield.

## 2. Materials and methods

### 2.1. Seedling production and *Bacillus* spp. treatment

Pepper, c.v. 'Camelot', seeds were sown in Cornell mix, a soilless peat mixture, with perlite and vermiculite (4:1:1) in 256- (2003) or 128 (2004 and 2005)-cell plug trays (Griffin Greenhouse and Nursery Supplies, Auburn, NY, USA) commonly used for pepper transplant production in New York, USA. Each tray was 42 cm × 25.5 cm, with a cell size of 1.5 cm × 1.5 cm or 2 cm × 2 cm for the 256- and 128-cell plug trays, respectively. Nitrogen, phosphorus, and potassium (10–5–10) fertilizer was added at a rate of 2.67 kg/m<sup>3</sup>.

The PGPR-containing product BioYield™ (Bayer CropScience LP, Research Triangle Park, NC, USA) was mixed with potting mix prior to planting (1.2 kg/m<sup>3</sup>). The formulation contains two bacterial strains, *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a. Plants were grown in a greenhouse under natural sunlight with temperatures of 23–26 °C (day) and 20–22 °C (night). In Geneva, NY, USA, the photoperiod is approximately 15/9 L/D from mid-May to mid-June. Prior to planting, seedlings were placed in an outdoor cold frame for 7 days to harden seedlings. Plants were fertilized with liquid fertilizer (15:30:15 N–P–K) prior to field planting.

### 2.2. *Bacillus* spp. impact on germination and seedling size

In the greenhouse, the germination rate (number of germinated seeds out of total seeds planted over time) was compared between plants grown in *Bacillus* spp.-treated and untreated potting mix in 2004 and 2005. In 2004, numbers of germinated seeds in each 128-cell plug tray were recorded twice per week for a month for a total of nine observations. In 2005, germination was recorded every 3–5 days for 3 weeks after sowing seed for a total of five observations.

Dry weight of 20 plants grown in either *Bacillus* spp.-treated or untreated potting mix was measured as described previously (Still and Pail, 2004), with a slight modification. Shoots and roots of 5-week-old plants were washed and dried separately, and tissue was dried in paper bags in a 65 °C oven for 5 days.

### 2.3. Field experiments to evaluate performance of *Bacillus* spp.

Field experiments were conducted at the New York State Agricultural Experiment Station's Fruit and Vegetable Research Farm in Geneva, NY, USA, from 2003 to 2005. In all experiments, 6-week-old transplants were hand-planted in the field on 17 June, 16 June, and 8 June, respectively. Seedlings were transplanted into beds covered with black plastic mulch with plants spaced at 30.5 cm intervals within the row. Each plot consisted of two 6.1 m rows, spaced 0.9 m apart with 20 plants per row. Peppers were fertilized, irrigated, and weeds controlled following typical production practices in western NY, USA (Reiners and Petzoldt, 2007).

### 2.4. Manipulating aphid densities in pepper using *esfenvalerate*

The ability to generate high populations of aphids was important to enable evaluation of the impact of *Bacillus* spp. on *M. persicae*. The premise behind this approach was to utilize an insecticide to which *M. persicae* populations would be resistant, whereas populations of natural enemies would be eliminated. In the absence of natural enemies, 0M. *persicae* populations would increase. To insure the

utility of this approach, field plots of peppers were either treated with a low rate of the broad-spectrum pyrethroid insecticide esfenvalerate (Asana XL, DuPont, Wilmington, DE, USA) or left untreated. Treatments were arranged in a randomized complete block design and replicated four times. Esfenvalerate was applied twice, on 30 July and 19 August, 2003 at a rate of 4.79 ml/ha, which is below the manufacturer's recommended rate. *M. persicae* populations were recorded weekly, five times during the months of August and September. Numbers of wingless aphids were visually assessed in the field and recorded from 20 randomly collected leaves from one row per plot. The plot row sampled was alternated each week to prevent unequal removal of foliage.

### 2.5. Impact of *Bacillus* spp. on aphid control

Aphid populations on pepper plants were compared among four treatments including: (1) plants grown in *Bacillus* spp.-treated potting mix (BioYield), (2) plants treated with the conventionally used systemic insecticide imidacloprid (Admire 2F, Bayer CropScience LP, Research Triangle Park, NC, USA), (3) plants grown in the presence of *Bacillus* spp. and treated with imidacloprid, and (4) untreated control plants. Treatments were arranged in a randomized complete block design with four replications. Imidacloprid was applied as an in-furrow drench at planting at a rate of 19.15 ml/ha. Each year, the entire test site was treated with a low rate (4.79 ml/ha) of esfenvalerate to increase *M. persicae* populations to allow evaluation of treatments under high aphid pressure. In 2003, applications were made on 30 July and 19 August, and in 2004 applications were made on 16 July, and 6 and 9 August. In 2005, one application was made on 25 July.

In 2003 and 2004, numbers of wingless *M. persicae* were assessed five and four times, respectively, in August and September. Densities were recorded using the same method described above.

### 2.6. Impact of *Bacillus* spp. on fruit yield

All fruit greater than 5 cm in diameter were harvested, counted, and weighed during each of the three seasons, from each treatment. In 2003, plots were harvested on 11 and 18 September; in 2004 plots were harvested on 20 August, and 3 and 21 September; and in 2005 plots were harvested on 19 August and 8 September. Yield comparisons among treatments were made at each harvest date, as well as for the total season yield for each treatment.

### 2.7. Statistical analyses

All data were analyzed using SAS software version 9.1 (Cary, NC, USA). Numbers of germinated seedlings over time were analyzed for interactions between time and treatment using a univariate mixed-effects analysis of

variance (Proc Mixed) and evaluated using least-squares means. Percentage of germinated seed at each time point, root and shoot dry weights, aphid densities, and yield (fruit number and weight) were assessed using a one-way analysis of variance (Proc GLM), and means compared with a Fisher's protected LSD at  $P < 0.05$ . For analysis of aphid densities and season aphid totals, this technique was preferred over a repeated measures analysis of variance because the study was not designed to compare population trends among treatments. To stabilize variance in aphid data sets prior to analysis, the transformation  $\log_{10}(x + 1)$  was used. Untransformed means are presented.

## 3. Results

### 3.1. Impact of *Bacillus* spp. on germination and seedling development

The *Bacillus* spp.-treated potting mix did not affect germination or enhance seedling development. In 2004 and 2005, the rate of germination was not significantly different between treated and untreated control plants ( $F = 1.17$ ;  $df = 1, 221$ ;  $P = 0.19$ ,  $F = 0.19$ ;  $df = 1, 33$ ;  $P = 0.67$ , respectively). In 2004, average germination rates were  $1.22 \pm 0.07$  (untreated) and  $1.26 \pm 0.08$  (BioYield) seedlings per day, while in 2005 rates were  $4.49 \pm 0.05$  (untreated) and  $4.52 \pm 0.07$  (BioYield) seedlings per day. Additionally, there were no significant differences in numbers of germinated seed at any recording time in either year ( $F = 1.25$ ;  $df = 1, 221$ ;  $P = 0.27$ ,  $F = 0.23$ ;  $df = 1, 33$ ;  $P = 0.64$ , respectively). In 2004, there was a very low percent germination in untreated and *Bacillus* spp.-treated potting mix ( $29.8 \pm 0.02\%$  and  $30.3 \pm 0.02\%$ , respectively), while in 2005 a much higher percent germination was observed at  $98.7 \pm 0.01\%$  for untreated and  $97.4 \pm 0.01\%$  for *Bacillus* spp.-treated mix.

Five weeks after planting, pepper root and shoot dry weights did not differ between treatments (root weight:  $F = 1.58$ ,  $df = 20$ ,  $P = 0.16$ ; shoot weight:  $F = 1.11$ ,  $df = 20$ ,  $P = 0.41$ ). Peppers grown in the presence of *Bacillus* spp. had an average shoot dry weight of  $0.87 \pm 0.05$  g, while shoots of untreated plants averaged  $0.80 \pm 0.05$  g. Root dry weight for *Bacillus*-treated peppers and untreated peppers averaged  $0.41 \pm 0.03$  and  $0.42 \pm 0.03$  g, respectively.

### 3.2. Effect of esfenvalerate on aphid densities

The season total number of aphids in plots treated with esfenvalerate was 2022 aphids per 20 leaves, five times greater than the total number in the untreated control ( $F = 15.25$ ;  $df = 1, 3$ ;  $P = 0.03$ ) (Table 1). A dramatic separation in aphid densities between treatments occurred on 3 and 9 September, 2 and 3 weeks after the second application of esfenvalerate, respectively (Table 1).

Table 1  
Effect of esfenvalerate on wingless populations of *Myzus persicae* on bell pepper in NY, USA, in 2003

Treatment	Mean number ± SE of wingless aphids per 20 leaves <sup>a</sup>					
	18 August	25 August	3 September	9 September	17 September	Season total <sup>b</sup>
Untreated	101 ± 29	106 ± 30	126 ± 61	97 ± 52	27 ± 13	457 a
Esfenvalerate <sup>c</sup>	44 ± 6	175 ± 30	900 ± 342	851 ± 175	48 ± 28	2022 b

<sup>a</sup>Data were transformed by  $\log_{10}(x+1)$  before analysis, but untransformed means are presented.

<sup>b</sup>Means within a column followed by different letters are significantly different ( $P > 0.05$ , Fisher's LSD).

<sup>c</sup>Esfenvalerate treatments were applied on 30 July and 19 August. Few aphids were observed at the test site in late July, and therefore were not recorded.

Table 2  
Effects of *Bacillus* species and imidacloprid on wingless populations of *Myzus persicae* on bell pepper in NY, USA, in 2003 and 2004

2003 Treatment <sup>a</sup>	Mean number ± SE of wingless aphids per 20 leaves <sup>b</sup>					
	18 August	25 August	3 September	9 September	17 September	Season total <sup>c</sup>
Control	44 ± 6	175 ± 30	900 ± 342	851 ± 175	48 ± 28	2022 a
<i>Bacillus</i> spp.	31 ± 8	122 ± 45	323 ± 143	567 ± 315	36 ± 24	1079 a
Imidacloprid	1 ± 1	1 ± 1	2 ± 1	2 ± 1	0 ± 0	6 b
2004 treatment <sup>a</sup>	Mean number ± SE of wingless aphids per 20 leaves <sup>b</sup>					
	27 August	3 September	10 September	20 September	Season total <sup>c</sup>	
Control	132 ± 56	135 ± 59	31 ± 11	5 ± 3	303 a	
<i>Bacillus</i> spp.	57 ± 9	102 ± 43	17 ± 8	4 ± 1	179 a	
Imidacloprid	2 ± 1	2 ± 1	1 ± 1	1 ± 0	5 b	
Imidacloprid + <i>Bacillus</i> spp.	0 ± 0	1 ± 0	0 ± 0	1 ± 0	2 b	

<sup>a</sup>Esfenvalerate was applied to all treatments, including the control on 30 July and 19 August 2003, and 16 July, 6, and 9 August 2004.

<sup>b</sup>Data were transformed by  $\log_{10}(x+1)$  before analysis, but untransformed means are presented.

<sup>c</sup>Means within a column followed by the same letter are not significantly different ( $P > 0.05$ , Fisher's LSD).

### 3.3. Efficacy of *Bacillus*-induced resistance to control green peach aphids

Peppers grown in the presence of *Bacillus* spp. had fewer aphids compared with the control, although not significantly fewer, in both 2003 and 2004 (Table 2). In contrast, imidacloprid provided excellent control of *M. persicae* in both years (2003:  $F = 18.19$ ,  $df = 5$ ,  $P = 0.001$ ; 2004:  $F = 19.53$ ,  $df = 5$ ,  $P = 0.0001$ ) (Table 2). In 2004, the combination of imidacloprid and *Bacillus* spp. did not provide a greater level of control than that provided by imidacloprid alone (Table 2).

Infestations of *M. persicae* were much higher in 2003 than in 2004 (Table 2). In 2003, the mean number of aphids per leaf during peak infestation in control plots was 900, only 2.7 times greater than the 323 observed in *Bacillus* spp.-treated plots, but 450 times greater than the mean number (2 aphids) in imidacloprid-treated plots. In 2004, the mean number of aphids per leaf during peak infestation in control plots was 135, only 1.3 times greater than the 102 found in *Bacillus*-spp.-treated plots, but 90 times greater than the 2 or 1 observed in imidacloprid-treated plots. In 2005, the test site was not colonized by sufficient numbers of *M. persicae* to establish a pest infestation. The

population was so low throughout the season that data were not collected.

### 3.4. Impact of *Bacillus* spp. on yield

**2003 Yield Study:** The majority of fruit from the 2003 season was collected during the final harvest on 18 September. In the initial harvest (11 September), mean number of fruit did not differ among treatments ( $P > 0.05$ ) (Table 3). Yet, peppers grown in *Bacillus* spp.-treated potting mix weighed significantly more than control and imidacloprid-treated peppers ( $F = 7.00$ ;  $df = 5$ ;  $P = 0.02$ ) (Table 3). In the final harvest, yield was significantly different between all treatments; yield was highest in imidacloprid-treated peppers and lowest in the untreated control (number of fruit:  $F = 21.37$ ,  $df = 5$ ,  $P = 0.001$ ; weight of fruit:  $F = 12.01$ ,  $df = 5$ ,  $P = 0.004$ ) (Table 3). The season total number and weight of fruit were greater in treated plots than the control, but did not differ significantly among imidacloprid and *Bacillus* spp. treatments (number of fruit:  $F = 11.34$ ,  $df = 5$ ,  $P = 0.005$ ; weight of fruit:  $F = 12.09$ ,  $df = 5$ ,  $P = 0.004$ ) (Table 3).

**2004 Yield Study:** Peppers were harvested three times throughout the 2004 season, with a very small initial harvest on 20 August. No difference between treatments

Table 3  
Effects of *Bacillus* species and imidacloprid insecticide on number and weight of bell pepper fruit at NY, USA, in 2003

Treatments <sup>a</sup>	1st harvest 11 September		2nd harvest 18 September		Total yield	
	Mean no. of fruit <sup>b,c</sup>	Mean weight (kg/plot)	Mean no. of fruit	Mean weight (kg/plot)	Mean no. of fruit	Mean weight (kg/plot)
Control	20.8 a	3.3 b	73.3 c	9.2 c	94.0 b	12.5 b
<i>Bacillus</i> spp.	32.5 a	6.6 a	98.3 b	11.8 b	130.8 a	18.4 a
Imidacloprid	29.5 a	4.6 b	127.3 a	15.5 a	156.8 a	20.0 a

<sup>a</sup>Esfenvalerate was applied to all treatments, including the control on 30 July and 19 August to increase the population of *M. persicae*.

<sup>b</sup>Means calculated based on 40 plants per plot.

<sup>c</sup>Means within a column sharing the same letters are not significantly different ( $P > 0.05$ , Fisher's LSD).

Table 4  
Effects of *Bacillus* species, imidacloprid, and both on total bell pepper yield at NY, USA, in 2004 and 2005

Treatment <sup>a</sup> 2004	1st harvest 20 August		2nd harvest 3 September		3rd harvest 21 September		Total yield	
	Mean no. of fruit <sup>b,c</sup>	Mean weight (kg/plot)	Mean no. of fruit	Mean weight (kg/plot)	Mean no. of fruit	Mean weight (kg/plot)	Mean no. of fruit	Mean weight (kg/plot)
Control	5.0 a	0.8 a	80.8 a	10.7 a	96.5 a	12.1 a	182.3 a	23.5 a
<i>Bacillus</i> spp.	5.3 a	0.9 a	56.8 a	7.1 a	66.8 ab	9.1 a	128.8 a	17.1 a
Imidacloprid	3.5 a	0.6 a	55.5 a	7.5 a	64.5 b	9.1 a	123.5 a	17.3 a
Imidacloprid + <i>Bacillus</i> spp.	4.8 a	0.8 a	56.5 a	7.3 a	76.3 ab	10.4 a	137.5 a	18.5 a
2005 Treatment <sup>a</sup>	1st harvest 19 August		2nd harvest 8 September		Total yield			
	Mean no. of fruit <sup>b,c</sup>	Mean weight (kg/plot)	Mean no. of fruit	Mean weight (kg/plot)	Mean no. of fruit	Mean weight (kg/plot)		
Control	60.3 a	6.7 a	43.3 a	5.0 a	103.5 a	11.7 a		
<i>Bacillus</i> spp.	60.5 a	7.1 a	46.0 a	5.4 a	106.5 a	12.5 a		
Imidacloprid	75.0 a	9.1 a	19.8 a	2.5 a	94.8 a	11.6 a		
Imidacloprid + <i>Bacillus</i> spp.	85.3 a	11.0 a	16.0 a	2.0 a	101.3 a	13.0 a		

<sup>a</sup>Esfenvalerate was applied to all treatments including the control on 16 July, 6, and 9 August 2004, and 25 July 2005 to increase the population of *M. persicae*.

<sup>b</sup>Means calculated based on 40 plants per plot.

<sup>c</sup>Means within a column sharing the same letters are not significantly different ( $P > 0.05$ , Fisher's LSD).

was observed in fruit number or weight in either of the first two harvests (20 August, 3 September), or for season totals (number of fruit:  $F = 2.4$ ,  $df = 6$ ,  $P = 0.11$ ; weight of fruit:  $F = 1.93$ ,  $df = 6$ ,  $P = 0.18$ ) (Table 4). In the third harvest, imidacloprid-treated plants had significantly less fruit than the untreated control, but did not differ significantly from the other treatments ( $F = 3.69$ ,  $df = 6$ ,  $P = 0.04$ ).

**2005 Yield Study:** The majority of peppers were harvested during the first of two harvests on 19 August. No difference between treatments was observed in fruit number or weight in either harvest ( $P > 0.05$ ) (Table 4). Total number and weight of fruit were not different among the treatments (number of fruit:  $F = 1.45$ ,  $df = 6$ ,  $P = 0.29$ ; weight of fruit:  $F = 1.97$ ,  $df = 6$ ,  $P = 0.17$ ) (Table 4).

#### 4. Discussion

Several studies have reported the utility of *Bacillus* PGPR species for growth promotion and biological control

of diseases of pepper (Kokalis-Burelle et al., 2002; Garcia et al., 2004; Joo et al., 2005; Russo, 2006), but our study is the first to examine the efficacy against *M. persicae* in addition to impact on growth promotion and yield. Previous research on pepper transplants grown in Florida found the mixture of *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a to increase transplant vigor (Kokalis-Burelle et al., 2002). The same mixture was used in the current study and found to have no impact on the rate of seed germination, or on early seedling growth. A difference in pepper varieties used in the two studies could explain this difference. Alternatively, the variation in growing conditions between the warmer Florida climate and that of the cooler climate in New York may have played a role in the increased vigor seen in the previous study compared with the present study. Two other previous reports of increased pepper plant growth following inoculation with PGPR utilized *Bacillus cereus* MJ-1 (Joo et al., 2005) and *Bacillus licheniformis* (Garcia et al., 2004). In these studies,

the difference in *Bacillus* spp. used could explain differences in results.

Our study followed germination rates in two seasons (2004 and 2005). The overall germination rate in 2004 was very low at approximately  $30 \pm 0.02\%$ . This low rate was due to problems with greenhouse heating combined with cool spring temperatures. Even though the overall rate was low, *Bacillus* spp.-treated and untreated planting mix had statistically similar germination rates. In 2005, germination rates were much higher (approximately  $98 \pm 0.01\%$ ), and again there were no significant differences between treatments. The germination rates between treatments were similar in each of the two seasons, with no difference observed between *Bacillus* spp.-treated and untreated planting mix. Therefore, we are confident that the low germination rate in 2004 did not have a greater impact on seed sown in treated versus untreated mix.

The ability to generate high populations of aphids was important for evaluating the impact of *Bacillus* spp. on *M. persicae*. Esfenvalerate is a broad-spectrum insecticide that at low rates is harsh on natural enemies, but does not harm *M. persicae* (Mowry, 2005). Esfenvalerate was found to be highly effective in 2003, with *M. persicae* populations increasing to significantly higher levels in treated plots. A delay in the population increase was observed, possibly due to a time delay between killing natural enemies (predators and parasitoids) of aphids and *M. persicae* reproduction. The final aphid collection of 2003 showed no difference in aphid levels between esfenvalerate and untreated plants. This was due to naturally declining aphid populations at season end. The rapid decline in aphid populations is frequently due to a decrease in aphid fecundity associated with declining plant quality or weather-induced mortality (Frazer, 1988). Applications of esfenvalerate were effective in causing populations of *M. persicae* to increase on pepper in 2003 and 2004, but not in 2005.

*M. persicae* populations were high in 2003, moderate in 2004, and did not develop on peppers in 2005. Environmental factors such as high levels of precipitation early in the season followed by excessively high air temperature and levels of solar radiation later in the season could be responsible, as these factors impact aphid population dynamics (Carver, 1988). In June 2005, there was 1224.28 cm of precipitation (a 65% increase from 2004, 37% increase from 2003). Additionally, the average high temperature for June, July, and August was  $27.9^\circ\text{C}$ , with 35 days over  $30^\circ\text{C}$ . There were only 8 and 5 days with high temperatures above  $30^\circ\text{C}$  in 2003 and 2004, respectively.

Growing plants in the presence of *Bacillus* PGPR did not control aphid infestations when *M. persicae* populations were present in 2003 and 2004. In contrast, imidacloprid was very successful in aphid control in both years. While the differences were not significant, there were fewer aphids colonizing plants grown in the presence of *Bacillus* PGPR compared with untreated control plants at each rating date in both 2003 and 2004. While the PGPR clearly do not control aphids to the level of imidacloprid, there is

certainly a trend toward fewer aphids on treated plants. Future studies may enable us to better understand these interactions.

Differences in yield between plants grown with PGPR, imidacloprid-treated, and control pepper plants were observed in 2003, with plants grown in the presence of *Bacillus* spp. producing fruit with a significantly greater weight in the first harvest on 11 September. This increase in fruit weight would be important in commercial production as a premium is paid for early fruit. By the second harvest on 18 September, the impact of heavy aphid pressure reduced the yield on both *Bacillus* spp.-treated and control plants compared with imidacloprid-treated plants. Interestingly, at this second harvest the *Bacillus* spp.-treated plants still had a significantly higher yield in both number and weight of fruit compared with the control plants. This increase in yield was not observed in plants grown in the presence of *Bacillus* spp. during the 2004 and 2005 seasons. This could be because the yield and growth benefits of this PGPR may only be seen under stressful conditions, such as high aphid pressure. Previous studies have shown PGPR to aid in plant responses to salt stress, drought, and phosphorus deficiency (Mayak et al., 2004; Wittenmyer and Merbach, 2005; Saravanakumar and Samiyappan, 2007).

We hypothesized that the *Bacillus* PGPR compound would improve plant growth, control *M. persicae* infestations, and increase pepper yield in New York, USA. Results indicated that the *Bacillus* PGPR had no statistically significant effect on germination, seedling growth, or control of *M. persicae*. Under high aphid pressure, as observed in 2003, plants grown in the presence of *Bacillus* PGPR tolerated economically damaging levels of *M. persicae* without a reduction in yield. Thus, it is possible that *Bacillus* PGPR could be useful in a *M. persicae* management program for pepper plants grown in locations with consistently high aphid pressure, though subsequent research in years of high aphid pressure would be necessary to fully support this conclusion. In a pepper management scheme, utilization of a broad-spectrum product to control European corn borer, *Ostrinia nubilalis* Hübner, infestations could cause *M. persicae* populations to increase rapidly. However, control of aphids may not be necessary if a *Bacillus* PGPR is used. Moreover, the combination of *Bacillus* PGPR and conservation of naturally occurring biological control organisms in pepper fields may further suppress aphid populations and preclude the need for aphid control using insecticides.

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## References

- Antoun, H., Prevost, D., 2006. Ecology of plant growth promoting rhizobacteria. In: Siddiqui, Z.A. (Ed.), *PGPR: Biocontrol and Biofertilization*. Springer, Dordrecht, pp. 1–38.
- Bostock, R.M., 2005. Signal crosstalk and induced resistance: straddling the line between cost and benefit. *Annu. Rev. Phytopathol.* 43, 545–580.
- Boughton, A.J., Hoover, K., Felton, G.W., 2006. Impact of chemical elicitor applications on greenhouse tomato plants and population growth of the green peach aphid, *Myzus persicae*. *Entomol. Exp. Appl.* 120 (3), 175–188.
- Bowen, G.D., Rovira, A.D., 1999. The rhizosphere and its management to improve plant growth. *Adv. Agron.* 66, 1–102.
- Carver, M., 1988. Biological control of aphids. In: Minks, A.K., Harrewijn, P. (Eds.), *Aphids: Their Biology, Natural Enemies, and Control*, vol. 2C. Elsevier Science Publishers, Amsterdam, pp. 141–165.
- Conrath, U., Beckers, G.J.M., Flors, V., Garcia-Agustin, P., Jakab, G., Mauch, F., Newman, M.A., Pieterse, C.M.J., Poinssot, B., Pozo, M.J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L., Mauch-Mani, B., Grp, P.-A.-P., 2006. Priming: getting ready for battle. *Mol. Plant Microbe Interact.* 19 (10), 1062–1071.
- Devonshire, A.L., 1989. Resistance of aphids to insecticides. In: Minks, A.K., Harrewijn, P. (Eds.), *Aphids: Their Biology, Natural Enemies, and Control*, vol. 2C. Elsevier Science Publishers, Amsterdam, pp. 123–139.
- Frantz, J.A., Gardner, J., Hoffmann, M.P., Jahn, M.M., 2004. Greenhouse screening of *Capsicum* accessions for resistance to green peach aphids (*Myzus persicae*). *Hortscience* 39 (6), 1332–1335.
- Frazer, B.D., 1988. Predators. In: Minks, A.K., Harrewijn, P. (Eds.), *Aphids: Their Biology, Natural Enemies and Control*, vol. 2B. Elsevier Science Publishers, Amsterdam, pp. 217–230.
- Garcia, J.A.L., Probanza, A., Ramos, B., Palomino, M.R., Manero, F.J.G., 2004. Effect of inoculation of *Bacillus licheniformis* on tomato and pepper. *Agronomie* 24 (4), 169–176.
- Joo, G.J., Kim, Y.M., Kim, J.T., Rhee, I.K., Kim, J.H., Lee, I.J., 2005. Gibberellins-producing rhizobacteria increase endogenous gibberellins content and promote growth of red peppers. *J. Microbiol.* 43 (6), 510–515.
- Kempster, V.N., Scott, E.S., Davies, K.A., 2002. Evidence for systemic, cross-resistance in white clover (*Trifolium repens*) and annual medic (*Medicago truncatula* var *truncatula*) induced by biological and chemical agents. *Biocontrol Sci. Technol.* 12 (5), 615–623.
- Kennedy, I.R., Choudhury, A.T.M.A., Kecskes, M.L., 2004. Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? *Soil Biol. Biochem.* 36 (8), 1229–1244.
- Kloepper, J.W., Ryu, C.M., Zhang, S.A., 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* 94 (11), 1259–1266.
- Kokalis-Burelle, N., Vavrina, C.S., Roskopf, E.N., Shelby, R.A., 2002. Field evaluation of plant growth-promoting rhizobacteria amended transplant mixes and soil solarization for tomato and pepper production in Florida. *Plant Soil* 238 (2), 257–266.
- Mayak, S., Tirosh, T., Glick, B.R., 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem.* 42 (6), 565–572.
- Minks, A.K., Harrewijn, P. (Eds.), 1989. *Aphids: Their Biology, Natural Enemies, and Control*, vol. C. Elsevier Science Publishers, Amsterdam.
- Mowry, T.M., 2005. Insecticidal reduction of potato leafroll virus transmission by *Myzus persicae*. *Ann. Appl. Biol.* 146 (1), 81–88.
- Murphy, J.F., Zehnder, G.W., Schuster, D.J., Sikora, E.J., Polston, J.E., Kloepper, J.W., 2000. Plant growth-promoting rhizobacterial mediated protection in tomato against *Tomato mottle virus*. *Plant Dis.* 84 (7), 779–784.
- Nelson, L. M., 2004. Plant growth promoting rhizobacteria (PGPR): prospects for new inoculants. *Crop Manage.* doi:10.1094/CM-2004-0301-05-RV.
- Persello-Cartieaux, F., Nussaume, L., Robaglia, C., 2003. Tales from the underground: molecular plant–rhizobacteria interactions. *Plant Cell Environ.* 26 (2), 189–199.
- Pieterse, C.M.J., Van Wees, S.C.M., Ton, J., Van Pelt, J.A., Van Loon, L.C., 2002. Signalling in rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. *Plant Biol.* 4 (5), 535–544.
- Reiners, S., Petzoldt, C. (Eds.), 2007. *The Integrated Crop and Pest Management Guidelines for Vegetables*. Cornell University, Ithaca.
- Russo, V.M., 2006. Biological amendment, fertilizer rate, and irrigation frequency for organic bell pepper transplant production. *Hortscience* 41 (6), 1402–1407.
- Ryu, C.M., Hu, C.H., Reddy, M.S., Kloepper, J.W., 2003. Different signaling pathways of induced resistance by rhizobacteria in *Arabidopsis thaliana* against two pathovars of *Pseudomonas syringae*. *New Phytol.* 160 (2), 413–420.
- Saravanakumar, D., Samiyappan, R., 2007. ACC deaminase from *Pseudomonas fluorescens* mediated saline resistance in groundnut (*Arachis hypogaea*) plants. *J. Appl. Microbiol.* 102 (5), 1283–1292.
- Still, J.R., Pill, W.G., 2004. Growth and stress tolerance of tomato seedlings (*Lycopersicon esculentum* Mill.) in response to seed treatment with paclobutrazol. *J. Hortic. Sci. Biotechnol.* 79 (2), 197–203.
- Stout, M.J., Thaler, J.S., Thomma, B.P.H.J., 2006. Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. *Annu. Rev. Entomol.* 51, 663–689.
- Stout, M.J., Zehnder, G.W., Baur, M.E., 2002. Potential for the use of elicitors of plant defense in arthropod management programs. *Arch. Insect Biochem.* 51 (4), 222–235.
- Tuzun, S., Bent, E. (Eds.), 2006. *Multigenic and Induced Systemic Resistance in Plants*. Springer Science+Business Media, Inc., New York.
- Wang, K.Y., Liu, T.X., Yu, C.H., Jiang, X.Y., Yi, M.Q., 2002. Resistance of *Aphis gossypii* (Homoptera: Aphididae) to fenvalerate and imidacloprid and activities of detoxification enzymes on cotton and cucumber. *J. Econ. Entomol.* 95 (2), 407–413.
- Wittenmyer, L., Merbach, W., 2005. Plant responses to drought and phosphorus deficiency: contribution of phytohormones in root-related processes. *J. Plant Nutr. Soil Sci.* 168 (4), 531–540.
- Zehnder, G., Kloepper, J., Tuzun, S., Yao, C.B., Wei, G., Chambliss, O., Shelby, R., 1997. Insect feeding on cucumber mediated by rhizobacteria-induced plant resistance. *Entomol. Exp. Appl.* 83 (1), 81–85.
- Zehnder, G.W., Murphy, J.F., Sikora, E.J., Kloepper, J.W., 2001. Application of rhizobacteria for induced resistance. *Eur. J. Plant Pathol.* 107 (1), 39–50.