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### 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, Acyrthosiphon 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 Bacilluscontaining 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  $42 \, \text{cm} \times 25.5 \, \text{cm}$ , with a cell size of  $1.5 \, \text{cm} \times 1.5 \, \text{cm}$  or  $2 \, \text{cm} \times 2 \, \text{cm}$  for the 256- and 128-cell plug trays, respectively. Nitrogen, phosphorus, and potassium (10-5-10) fertilizer was added at a rate of  $2.67 \, \text{kg/m}^3$ .

The PGPR-containing product BioYield <sup>TM</sup> (Bayer CropScience LP, Research Triangle Park, NC, USA) was mixed with potting mix prior to planting (1.2 kg/m³). 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 Pill, 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 handplanted 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, 0*M. persicae* populations would increase. To insure the

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