

# Plant Growth-Promoting Rhizobacterial Mediated Protection in Tomato Against *Tomato mottle virus*

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## ABSTRACT

Murphy, J. F., Zehnder, G. W., Schuster, D. J., Sikora, E. J., Polston, J. E., and Kloepper, J. W. 2000. Plant growth-promoting rhizobacterial mediated protection in tomato against *Tomato mottle virus*. *Plant Dis.* 84:779-784.

Tomato plants treated with plant growth-promoting rhizobacteria (PGPR), applied as an industrially formulated seed treatment, a spore preparation mixed with potting medium (referred to as powder), or a combined seed-powder treatment, were evaluated under field conditions for induced resistance to *Tomato mottle virus* (ToMoV). The PGPR strains used, based on their ability to induce resistance in previous experiments, included *Bacillus amyloliquefaciens* 937a, *B. subtilis* 937b, and *B. pumilus* SE34. Experiments were conducted in the fall of 1997 and the spring and fall of 1998 at the University of Florida's Gulf Coast Research & Education Center, Bradenton. All plants were rated for symptoms and analyzed for the presence of ToMoV DNA at 40 days after transplant (dat). Whitefly densities were determined on individual plants in each trial, and marketable fruit yields were determined at least two times during each trial. The highest level of protection occurred in the fall 1997 trial when, at 40 dat, ToMoV disease severity ratings were significantly less in all PGPR powder-based treatments than in either of the seed or control treatments. Detection of viral DNA using Southern dot blot analyses correlated with symptom severity ratings, as did fruit yields. A reduction in ToMoV symptom severity ratings and incidence of viral DNA were also observed for some PGPR treatments in the spring 1998 trial, although corresponding yield responses were not apparent. Little or no resistance was observed in the fall 1998 trial. No differences in disease severity, detection of ToMoV DNA, or yield occurred among treatments in any of the trials at 80 dat. These data show that up to 40 dat under natural conditions of high levels of vector-virus pressure, some PGPR treatments resulted in reduced ToMoV incidence and disease severity and, in some cases, a corresponding increase in fruit yield. The use of PGPR could become a component of an integrated program for management of this virus in tomato.

Additional keywords: biocontrol, geminivirus

Plant virus disease management strategies typically include the use of genetically resistant varieties, the integration of selected cultural practices, the application of insecticides to control insects that might serve as vectors, and combinations thereof (5). Two additional approaches to managing viruses include cross protection and development of genetically engineered plants that express a viral structural or nonstructural protein (4,6,11,31). The use of genetically resistant varieties is clearly the most economically and environmentally sound choice; however, commercially acceptable varieties that resist virus infection are not always available. Cross pro-

tection has been used successfully with several virus-host systems (9), but this approach is not feasible with some crops, and there are obvious risks associated with inoculation of a crop with an infectious agent. Genetically engineered crops should serve to selectively target viruses for which resistant varieties are not available; however, to date, virus resistant transgenic squash is the only example commercially available (29).

The efficacy of reducing virus infection via control of its vector through application of insecticides is dependent on the mode of transmission (e.g., it is generally ineffective with viruses transmitted in a nonpersistent manner). To be effective, this approach requires timely insecticidal applications based on knowledge of vector ecology within a given area. The application of insecticides, however, also has associated environmental concerns.

Management of a viral disease can also be accomplished through the induction of a plant's natural defenses, e.g., systemic

acquired resistance (SAR; 24). SAR against viral infection has been documented using biological and chemical inducing agents (1,2,7,10,12,16,21,23). In most cases, the biological agents consisted of plant pathogenic bacteria, fungi, or viruses.

An alternative method to induce plant defenses is through the use of nonpathogenic microorganisms. This approach has been referred to as induced systemic resistance (30). Mann (12) applied cultures of *Bacillus uniflagellatus* and extracts from such cultures to tobacco roots as soil drenches in an attempt to induce systemic resistance to *Tobacco mosaic virus* (TMV). Each treatment resulted in a significant reduction in the number of lesions resulting from TMV infection. Maurhofer et al. (14) evaluated the root-colonizing bacterium *Pseudomonas fluorescens* as an inducing agent against the lesion-inducing *Tobacco necrosis virus* (TNV) in tobacco. They also observed a reduction in TNV-induced lesion number in *P. fluorescens*-treated plants. Raupach et al. (22) were the first to show that treatment of cucumber or tomato plants with plant growth-promoting rhizobacteria (PGPR) resulted in induced systemic resistance against systemic infection by *Cucumber mosaic virus* (CMV). Zehnder et al. (33) identified PGPR strains that protected tomato against systemic infection by CMV under greenhouse and field conditions.

Whitefly-transmitted geminiviruses have emerged as a serious threat to vegetable production in the Western Hemisphere. In the early 1990s, an outbreak of *Tomato mottle virus* (ToMoV) threatened the tomato industry in Florida (8,15,27). ToMoV incidences as high as 100% were reported in tomato crops grown in Florida (19), with estimated losses of \$140 million in 1990 and 1991 (25). Since its identification in Florida, ToMoV has been detected in South Carolina, Tennessee, and Virginia (18). Management of whitefly-transmitted geminiviruses in tomato has been difficult due to a lack of genetically resistant varieties available for commercial use, the complexity of whitefly biology, and the ability of whiteflies to develop resistance to insecticides (3,28). Currently, the only effective control used in Florida is the application of the systemic insecticide imidaclo-

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prid, which has significantly reduced whitefly populations, and in turn, reduced the incidence of ToMoV and other whitefly-transmitted geminiviruses (17).

We have evaluated the efficacy of PGPR-mediated resistance in fresh-market tomato against ToMoV infection under field conditions. We show that treatment of tomato plants with selected PGPR strains in some cases reduced ToMoV disease severity and incidence of ToMoV, based on detection of ToMoV DNA by Southern dot blot analysis, within the first 40 days following transplant to the field. Furthermore, some of the PGPR treatments resulted in increased tomato fruit yield at the first of two or more harvests.

## MATERIALS AND METHODS

**Field design.** Three field trials, one each in fall 1997, spring 1998, and fall 1998, were conducted at the University of Florida's Gulf Coast Research & Education Center, Bradenton. Tomato plants were grown on 20-cm-high by 84-cm-wide beds of EauGallie fine sand. Prior to bedding, P<sub>2</sub>O<sub>5</sub> fertilizer was broadcast at 2.5 kg per 30.5 m of row, and after bedding 15-0-30 fertilizer was banded on the bed shoulders at 9.1 kg per 30.5 m of row. Before covering the beds with either black (spring experiment) or white (fall experiments) polyethylene plastic film, the beds were fumigated with methyl bromide at 392 kg/ha. Single-row treatment plots were replicated four times in a completely randomized design and consisted of 10 plants (fall 1997 and spring 1998) or 12 plants (fall 1998) transplanted 46 cm apart at least 14 days after fumigating on 15 September 1997 and 30 March and 9 September 1998. Plants were seep-irrigated, staked, and tied three times. Chlorothalonil (Bravo) or a combination of copper hydroxide (Kocide DF) and maneb (Manex) were applied weekly for managing bacterial and fungal foliar pathogens. Lepidopterous larvae were controlled with weekly applications of *Bacillus thuringiensis* (Matth) or spinosad (SpinTor).

**PGPR treatments.** For the fall 1997 trial, two PGPR strains were evaluated, *Bacillus amyloliquefaciens* 937a and *B. subtilis* 937b (13). PGPR strains 937b and *B. pumilus* SE34 (13) were used in the spring 1998 trial, and all three PGPR strains were used in the fall 1998 trial. These three PGPR strains were chosen based on a previous study showing that each induced systemic resistance in tomato against infection by CMV (33). Spore preparations of each PGPR strain were applied as seed treatments, as powder amendments to the planting medium, or as a combined seed and powder treatment (referred to as seed+powder treatment). All PGPR strains were applied at the rate of  $1 \times 10^7$  CFU/g of seed or planting medium, except that an additional rate of  $1 \times 10^8$  CFU/g was included for the 937b soil mix

treatment in the fall 1997 and spring 1998 trials. The powders were applied to Peat Lite plant medium (Speedling, Inc., Sun City, FL) by mixing the required amount of PGPR powder for 908 g of dry planting medium in 250 ml of deionized water. A portion of the solution was misted onto the surface of the planting medium, and the medium was then mixed thoroughly. The process was repeated until the entire suspension was applied. The seed treatment was prepared and applied by staff scientists at Gustafson, Inc. (Plano, TX). The powder formulation was also prepared at Gustafson but delivered to Bradenton, FL, for incorporation into the planting medium.

Tomato cv. Agriset was seeded into the planting medium in styrofoam seeding trays with 2.5 by 2.5 cm cells (Speedling) and maintained in the greenhouse. The seedlings were fertilized 1 week after emergence at 6 g per 3,785 ml of 20-20-20 soluble fertilizer with the minor elements (Southern Agricultural Insecticides, Inc., Palmetto, FL) and then grown for approximately 5 weeks before being transplanted to the field. No insecticides were applied to plants while maintained in the greenhouse.

**Inoculation of tomato plants with ToMoV.** The PGPR plots were inoculated by natural movement of viruliferous silverleaf whitefly adults, *Bemisia argentifolii* Bellows & Perring, from adjacent tomato germ plasm being developed for ToMoV resistance. The germ plasm had been inoculated previously in small greenhouses by suspending whitefly-infested and ToMoV-infected tomato plants in baskets above the seedlings for about 2 weeks. These infected plants were then planted in the field several weeks prior to the planting of the PGPR-treated tomatoes.

**ToMoV infection and whitefly assessments.** Each tomato plant was evaluated for ToMoV infection by rating symptom severity and by Southern dot blot analysis for detection of ToMoV DNA at 40 (fall 1997, spring 1998, fall 1998) and 80 (fall 1997) days after transplanting (dat) to the field. A general symptom severity rating, but no Southern dot blot analysis, was carried out at 80 dat for the spring and fall 1998 trials. Symptom ratings were based on a 5 point scale where 0 = no symptoms, 1 = mild mottling on young leaves, 2 = obvious mottling on leaves from at least one of the main stems, 3 = obvious mottling on leaves over most of the plant, 4 = obvious mottling on leaves and leaf distortion over the entire plant, and 5 = obvious mottling on leaves, leaf distortion, and severe stunting. In addition to the symptom rating, a sample consisting of three terminal leaflets was collected randomly from the upper canopy of each plant and tested for the presence of ToMoV DNA by Southern dot blot analysis (20). Blots were hybridized under conditions of high stringency with a probe consisting of the

[<sup>32</sup>P]dCTP-labeled ToMoV B component DNA using a High Prime DNA Labeling Kit (Boehringer Mannheim Corp., Indianapolis, IN). After hybridization, blots were rinsed under conditions of high stringency and exposed to Hyperfilm MP film (Amersham Pharmacia Biotech, Piscataway, NJ) for 1 to 6 h.

Whitefly densities on tomato plants were determined in each of the trials. In the fall 1997 trial, crawlers (first instars), sessile nymphs (second and third instars), and pupae (fourth instars) were counted on 27 September and 28 October (12 and 43 dat, respectively). In the spring 1998 trial, crawlers (first instars), sessile nymphs (second and third instars), and pupae (fourth instars) were counted on 1 June (63 dat), while in the fall 1998 trial, crawlers and sessile nymphs were counted on 22 October and 16 November (43 and 68 dat, respectively). In each case, numbers were determined from the terminal leaflet of the seventh or eighth leaf from the top of each of 10 plants per plot.

**Yield.** Tomato fruit from 10 plants per plot were harvested at least twice during each trial and separated into marketable and nonmarketable categories. Nonmarketable fruit were undersized or misshapen, or possessed physical defects such as catfacing or other blossom end abnormalities. Marketable fruit were size-graded by a machine as extra large ( $\geq 70$  mm diameter), large (63.5 to 70.6 mm), and medium (57.2 to 64.3 mm). The number and weight of fruit in each category were recorded. The weights (kg per plot) of marketable fruit were totaled in all size categories for analysis of each harvest.

**Statistical analysis.** Data were analyzed using ANOVA; LSD tests were performed to compare treatment means at the 5% level. All analyses were performed using the GLM procedure of SAS (SAS Institute, 1990, Cary, NC).

## RESULTS

**Fall 1997 trial (40 days after transplant).** A visual assessment of treatments indicated differences in ToMoV disease severity and incidence among treatments. All powder and seed+powder PGPR treatments resulted in significantly lower disease severity ratings than the control or seed treatments with PGPR strains 937a and 937b (Fig. 1). The disease severity ratings among powder-based PGPR treatments did not differ significantly. Thus, increasing the concentration of PGPR strain 937b within the formulation did not result in a corresponding decrease in disease severity. PGPR seed treatments did not significantly affect symptom severity ratings.

Southern dot blot analysis for the detection of ToMoV DNA corresponded with the symptom severity ratings, e.g., the percentage of tomato plants infected by ToMoV was lower in all powder-based

treatments compared with plants subjected to the seed treatment alone or the control treatment, although differences were not always significant (Fig. 2). All powder-based PGPR treatments, except 937b/powder ( $10^8$  CFU/g), had significantly fewer ToMoV-infected plants than were detected in seed treatments with PGPR strains 937a and 937b. In addition, treatment of tomato plants with either 937b as a powder ( $10^7$  CFU/g) or the combined seed+powder resulted in significantly fewer ToMoV-infected plants than the control treatment.

Marketable fruit weight data from the first harvest indicated that plants in each of the powder-based PGPR treatments produced higher yields than plants in either of the PGPR seed treatments or the control treatment (Fig. 3). This difference was significant for PGPR treatment 937b/powder ( $10^7$  CFU/g). Overall, tomato yields were lower in the second and third harvests. In the second harvest, yields for some PGPR treatments were significantly lower than for plants in the control treatment, whereas no yield differences were observed among treatments for the third harvest (data not shown).

At the 27 September (12 dat) evaluation, there were no significant differences among treatments in numbers of whitefly crawlers or pupae (Table 1). However, significantly fewer nymphs occurred on tomato plants treated with PGPR strain 937b as a seed treatment or with the powder treatments of 937a and 937b (both concentrations) compared with tomato plants in the control treatment. Additionally, the higher concentration of PGPR strain 937b as a powder formulation resulted in significantly fewer nymphs occurring on tomato plants compared with that same PGPR strain applied as a combined seed+powder treatment. No significant reductions in the number of whiteflies occurred among PGPR treatments at the 28 October (43 dat) evaluation, although there was a significant increase in the number of crawlers on the 937b/S-treated tomato plants.

**Spring 1998 trial (40 days after transplant).** In this trial, PGPR strain SE34 was used in place of strain 937a with the same application methods used in the fall 1997 trial. ToMoV symptom severity ratings were significantly lower for plants in the 937b/seed treatment and for those plants treated with SE34 either as a powder or the combined seed+powder compared with plants in the control treatment and PGPR treatments SE34/seed and 937b/powder ( $10^8$  CFU/g) (Fig. 1).

A significant reduction in ToMoV incidence, based on Southern dot blot analysis and relative to the control treatment, occurred only for PGPR treatments 937b/seed and SE34/seed+powder (Fig. 2). However, ToMoV incidence was also significantly lower in the SE34/seed+powder treatment than in the SE34/seed treatment.

Fruit yields obtained for the first harvest of the spring 1998 trial were lower than those observed for either of the fall trials (Fig. 3). A difference in fruit yield between PGPR treatments occurred only for the first harvest with plants treated with strain 937b as the seed+powder formulation producing significantly more marketable tomato fruit than plants from each of the other PGPR treatments, although not significantly more than plants in the control treatment. No differences in fruit yield were observed among treatments at either the second or third harvests (data not shown).

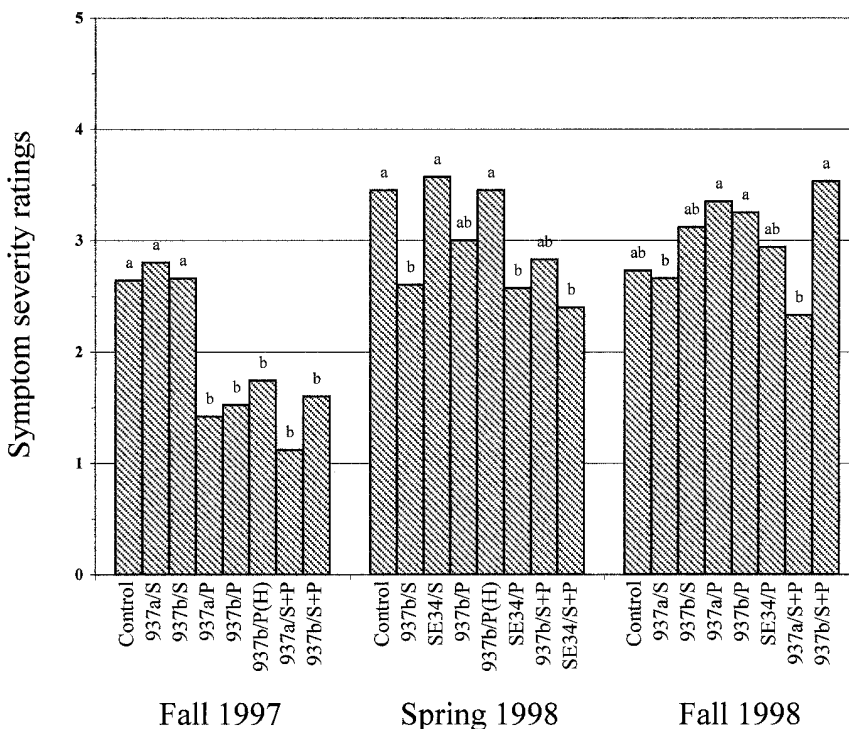
Significantly lower numbers of crawlers, nymphs, and pupae occurred on tomato plants in treatments 937b/powder ( $10^7$  CFU/g), SE34/powder, 937b/seed+powder, and SE34/seed+powder, relative to tomato plants in the control treatment (Table 1). In addition, a significantly lower number of pupae occurred on tomato plants in 937b/seed and SE34/seed treatments, with the 937b/seed treatment also resulting in a significant reduction in the number of nymphs relative to the control treatment.

**Fall 1998 trial (40 days after transplant).** All three PGPR strains were used in this trial, although SE34 was used only as a powder formulation. There were no

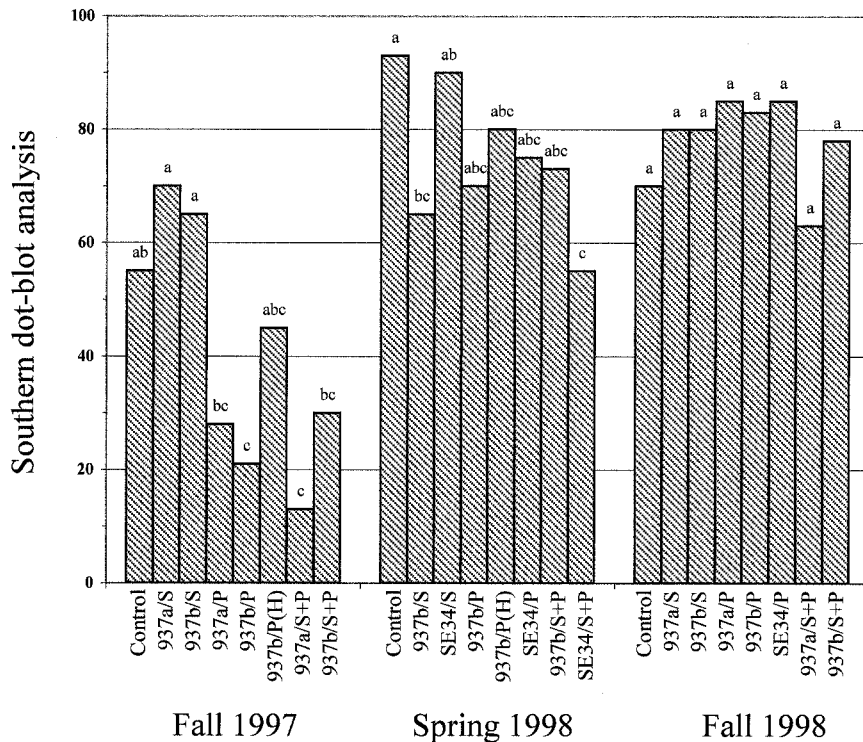
significant differences in ToMoV disease severity ratings between the PGPR treatments and the control treatment (Fig. 1). Only the seed and seed+powder treatments of strain 937a had a lower symptom severity rating than the control. The ToMoV disease severity rating for the seed and seed+powder treatments for strain 937a were significantly less than for the powder treatment of strain 937a and the powder and seed+powder treatments of strain 937b.

No significant differences in ToMoV disease incidence were observed between any of the treatments based on Southern dot blot analysis (Fig. 2).

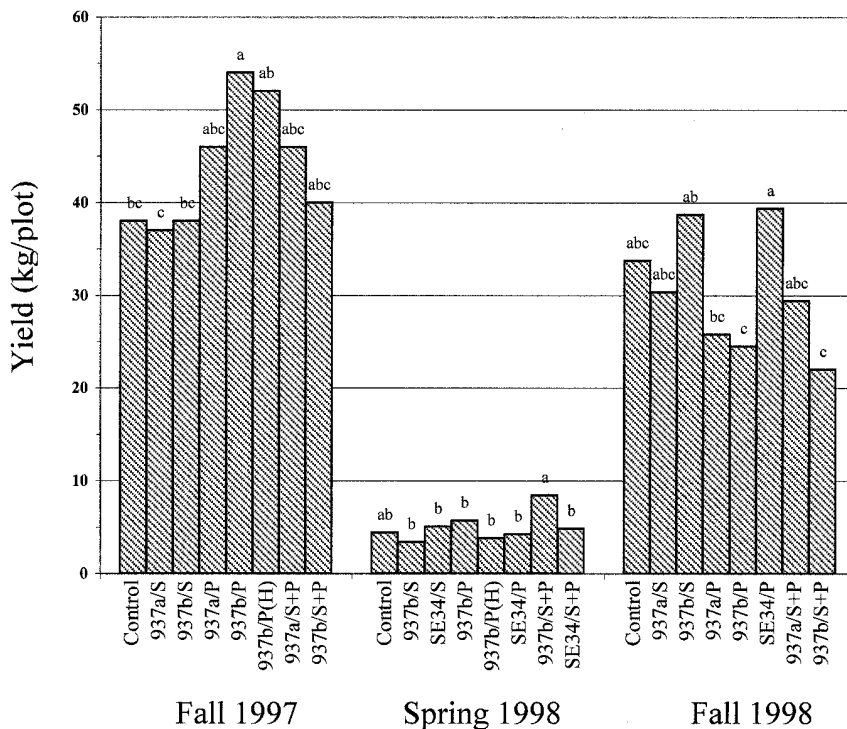
Marketable fruit yield for plants in the PGPR treatments did not differ significantly from those in the control treatment (Fig. 3). Tomato plants in the SE34/powder treatment produced higher yields than all other treatments; significantly higher than plants in the 937a/powder treatment and the powder and seed+powder treatments for strain 937b. Similarly, tomato plants in the 937b/seed treatment had higher yields than most other treatments; significantly higher than for plants treated with strain 937b as powder and seed+powder formulations. No differences in fruit yield oc-



**Fig. 1.** Tomato mottle virus disease severity ratings were determined for each tomato plant at 40 days after transplant in each of three trials (fall 1997, spring 1998, and fall 1998). Symptom ratings were based on a 0 to 5 point scale where 0 = no symptoms, 1 = mild mottling on young leaves, 2 to 5 = obvious mottling on leaves and (2) at least one of the main stems, (3) over most of the plant, (4) leaf distortion over the entire plant, and (5) leaf distortion and severe stunting. PGPR treatments listed along the x-axis included strains *Bacillus amyloliquefaciens* 937a, *B. subtilis* 937b, and *B. pumilus* SE34 as S = seed treatment, P = powder formulation of spores added to the planting medium, and S+P = combined seed+powder treatments. PGPR were applied at a rate of  $1 \times 10^7$  CFU/g with the exception of 937b/P(H), which was applied at a rate of  $1 \times 10^8$  CFU/g. Different letters represent a significant difference of the means at  $P = 0.05$  according to LSD tests.



**Fig. 2.** Percentage of tomato plants infected by *Tomato mottle virus* for each treatment as determined by Southern dot blot analysis. Foliar tissue was collected from each plant in each treatment at 40 days after transplant in each of three trials (fall 1997, spring 1998, and fall 1998) and analyzed according to Polston et al. (20). PGPR treatments listed along the x-axis included strains *Bacillus amyloliquefaciens* 937a, *B. subtilis* 937b, and *B. pumilus* SE34 as S = seed treatment, P = powder formulation of spores added to the planting medium, and S+P = combined seed+powder treatments. PGPR were applied at a rate of  $1 \times 10^7$  CFU/g with the exception of 937b/P(H), which was applied at a rate of  $1 \times 10^8$  CFU/g. Different letters represent a significant difference of the means at  $P=0.05$  according to LSD tests.



**Fig. 3.** Marketable tomato fruit yields (kg/plot) from 10 plants per plot from the first of two or three harvests for fall 1997, spring 1998, and fall 1998 trials. PGPR treatments listed along the x-axis included strains *Bacillus amyloliquefaciens* 937a, *B. subtilis* 937b, and *B. pumilus* SE34 as S = seed treatment, P = powder formulation of spores added to the planting medium, and S+P = combined seed+powder treatments. PGPR were applied at a rate of  $1 \times 10^7$  CFU/g with the exception of 937b/P(H), which was applied at a rate of  $1 \times 10^8$  CFU/g. Different letters represent a significant difference of means at  $P=0.05$  according to LSD tests.

curred among treatments at the second and third harvests (data not shown).

Densities of whitefly crawlers, nymphs, and pupae did not differ among treatments at either of the two evaluations (data not shown).

**Evaluations at 80 days after transplant.** At the 80 dat evaluation for each of the trials, few differences in disease severity ratings were observed among treatments. Southern dot blot analysis of plants at 80 dat during the fall 1997 trial substantiated visual assessments indicating that most or all plants in each of the treatments were infected with ToMoV.

## DISCUSSION

We evaluated the efficacy of selected PGPR strains to induce systemic resistance to ToMoV under natural conditions of inoculation by the silverleaf whitefly vector. In addition, PGPR treatments were industrially formulated, thereby bringing this approach closer to commercial implementation. Evaluations focused on ToMoV infection, determined visually and by detection of viral DNA by Southern dot blot analysis, and on yield responses. Visual assessments correlated well with detection of ToMoV DNA.

Two of the PGPR treatments resulted in a statistically significant reduction in ToMoV disease severity in the fall 1997 and spring 1998 trials, and Southern dot blot analysis substantiated these results. Although protection was observed in these trials, there was no consistent trend in resistance induced by any particular PGPR strain or application method from one trial to another. The most impressive level of protection occurred in the fall 1997 trial where all powder-based PGPR treatments resulted in reduced disease severity and incidence of ToMoV. In this case, reductions associated with disease severity ratings in each of the powder-based treatments were significant, whereas ToMoV incidence, based on detection of viral DNA, was significantly lower than in the control treatment only for 937b/powder ( $10^7$  CFU/g) and 937a/seed+powder treatments.

Yield responses among treatments followed a similar trend to disease severity ratings and Southern dot blot analyses. Fruit yields from the fall 1997 trial were greater for the PGPR powder-based treatments than for the PGPR seed treatments or the control treatment. Furthermore, similar to the results obtained with disease severity ratings and ToMoV DNA detection at 40 dat versus 80 dat, differences in yield (i.e., increases in yield) were observed only at the first harvest. No differences were observed among treatments for season-total marketable yields in any of the trials, suggesting that, although PGPR treatments may have enhanced yields at the first harvest, subsequent yields were equally affected by ToMoV. However, the

first harvest is often more economically important to growers, especially in the fall crop in west-central Florida.

These findings clearly show that treatment of tomatoes with PGPR can result in protection against ToMoV under natural conditions. The mechanism by which PGPR treatments reduced ToMoV-induced symptoms and incidence was not determined but could include resistance against ToMoV, resistance against the whitefly vector, or resistance against transmission of ToMoV by the whitefly vector. Resistance against ToMoV could occur at any of several stages in the infection process. Since symptom severity ratings and ToMoV incidence were reduced at 40 dat but not at 80 dat, a delay may have resulted from reduced accumulation of virus, systemic infection, or some combination thereof. In addition, test plants were continuously being attacked by increasing numbers of viruliferous whitefly adults migrating from the adjacent tomato germ plasm, and therefore, the induced resistance may have been overwhelmed. In a previous study, PGPR-treated cucumber plants mechanically inoculated with CMV did not develop symptoms, and no virus was detected in noninoculated leaves by ELISA (22). It was not determined whether CMV accumulated in the inoculated leaf and to what extent it may have moved cell-to-cell within the inoculated leaf.

In that same study, inoculation of PGPR-treated tomato plants with CMV resulted in reduced symptom severity compared with inoculated plants in the control treatment (22). This suggests that the level of PGPR-mediated resistance against a virus that systemically infects its host differs not only among PGPR strains but among plant species. Our results correspond with the observations of Raupach et al. (22); ToMoV symptoms developed on PGPR-treated tomato plants, but with reduced severity compared with the symptoms that developed in control plants.

Reduced whitefly densities occurred in the fall 1997 and spring 1998 trials. In the fall 1997 trial, nymph densities were significantly reduced in four of the PGPR treatments; three of these treatments included the 937a and 937b (both concentrations) powder formulations which also had reduced ToMoV disease severity and incidence and increased yields. This effect was more extensive in the spring 1998 trial, where crawler, nymph, and pupae densities were significantly reduced in four of the PGPR treatments. Furthermore, no differences in whitefly densities occurred on tomato plants among PGPR treatments in the fall 1998 trial, a trial that also had no significant differences in ToMoV disease severity, incidence, or yields. These observations strongly suggest that some PGPR treatments had a deleterious effect on whitefly densities; however, a correspond-

ing effect on ToMoV disease and tomato yields was inconsistent.

Whether the basis for the reduction in whitefly densities was due to deterrence, effects on developmental processes, or combinations thereof was not determined. If, however, the reduced whitefly densities on PGPR-treated tomato plants resulted in a lower dosage of ToMoV introduced into the plant, then a delay in the onset of symptoms and detection of ToMoV DNA may not be unexpected. Treatment of cucumber plants with PGPR resulted in reduced feeding by cucumber beetles and transmission of the bacterial wilt pathogen (32). The deterrent effect on beetle feeding was associated with a reduction in cucurbitacin (a plant secondary compound that acts as a feeding stimulant for cucumber beetles) in the PGPR-treated cucumber plants. The relationship between PGPR treatment and whitefly feeding on tomato was not investigated, and we did not determine whether the observed resistance was directed at ToMoV, transmission of ToMoV by the whitefly vector, or both. Our studies with PGPR-mediated resistance against CMV involved mechanical

inoculation of virus, indicating that the resistance was effective against some stage in the CMV infection process rather than interference in transmission (33).

Regardless of the basis for the protection that occurred in the PGPR-treated tomato plants, these data show that under natural conditions of high levels of vector-virus pressure, some PGPR treatments resulted in reduced ToMoV disease severity and incidence up to 40 dat. This is typically a critical time for effects on fruit production (26); i.e., yields are less affected if disease incidence and severity can be maintained at low levels through the first 40 dat. In this study, ToMoV incidence and disease severity may have been reduced in some PGPR treatments relative to plants in the control treatment; however, correlative responses in yield were not always observed, especially with the second and third harvests. This may stem from the fact that disease and vector pressure may have overwhelmed the PGPR-mediated resistance as the season progressed. Furthermore, in the spring 1998 and fall 1998 trials, other viruses, including potyviruses, closteroviruses, and *Tomato yellow leaf*

**Table 1.** Whitefly crawler, nymph, and pupa densities on tomato plants in the fall 1997 and spring 1998 trials

Treatment <sup>y</sup>	Crawlers	Nymphs	Pupae
Fall 1997			
27 September			
Control	13.7 a <sup>z</sup>	39.2 a	14.0 a
937a/S	14.0 a	26.0 abc	16.7 a
937b/S	7.5 a	16.7 bc	6.0 a
937a/P	6.2 a	18.2 bc	6.0 a
937b/P	11.5 a	18.7 bc	2.5 a
937b/P(H)	8.2 a	14.0 c	2.7 a
937a/S+P	10.2 a	23.2 abc	9.5 a
937b/S+P	11.2 a	36.0 ab	13.5 a
28 October			
Control	26.5 b	10.0 a	6.0 a
937a/S	12.7 b	12.0 a	3.2 a
937b/S	50.7 a	12.7 a	13.7 a
937a/P	18.0 b	10.5 a	11.0 a
937b/P	13.2 b	10.7 a	3.5 a
937b/P(H)	12.7 b	5.7 a	3.2 a
937a/S+P	20.2 b	14.2 a	10.7 a
937b/S+P	31.5 ab	9.7 a	4.0 a
Spring 1998			
1 June			
Control	39.6 a	48.7 a	54.7 a
937b/S	18.3 ab	19.7 b	21.3 b
SE34/S	23.3 ab	28.3 ab	18.3 b
937b/P	9.0 b	17.0 b	19.0 b
937b/P(H)	31.0 ab	37.3 ab	36.3 ab
SE34/P	8.0 b	21.0 b	15.7 b
937b/S+P	11.3 b	15.3 b	13.7 b
SE34/S+P	7.7 b	16.3 b	18.0 b

<sup>y</sup> Treatments included plant growth-promoting rhizobacteria (PGPR) strains *Bacillus amyloliquefaciens* 937a, *B. subtilis* 937b, and *B. pumilus* SE34 as S = seed treatment, P = powder formulation of spores added to the planting medium and S+P = combined seed+powder treatments. PGPR were applied at  $1 \times 10^7$  CFU/g with the exception of 937b/P(H), which was applied at a rate of  $1 \times 10^8$  CFU/g.

<sup>z</sup> Numbers represent mean values of whitefly crawlers (first instars), sessile nymphs (second and third instars), and pupae (fourth instars) counted on 27 September and 28 October (12 and 43 days after transplant, respectively) in the fall 1997 trial and on 1 June (63 days after transplant) in the spring 1998 trial. In each case, numbers were determined from the terminal leaflet of the seventh or eighth leaf from the top of each of 10 plants per plot. Means followed by the same letter within a column are not significantly different at  $P = 0.05$ .

*curl virus* were identified in the location where these studies were carried out and may have contributed to a lack in positive yield response to PGPR treatment (J. E. Polston, unpublished data). Nevertheless, the reduced disease severity and incidence of ToMoV resulting from the use of PGPR suggest that PGPR-mediated resistance has potential to become a component of an integrated program for management of this virus in tomato.

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