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Application of rhizobacteria for induced resistance

Geoffrey W. Zehnder[1], John F. Murphy[2], Edward J. Sikora[2] and Joseph W. Kloepper[2]
[1]*Department of Entomology, 114 Long Hall, Clemson University, Clemson, SC 29634, USA (Fax: +1 8646565065; E-mail: zehnder@clemson.edu);* [2]*Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849, USA*

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Abstract

This article provides a review of experiments conducted over a six-year period to develop a biological control system for insect-transmitted diseases in vegetables based on induced systemic resistance (ISR) mediated by plant growth-promoting rhizobacteria (PGPR). Initial experiments investigated the factors involved in PGPR induced resistance to bacterial wilt disease in cucumber caused by *Erwinia tracheiphila*. Results demonstrated that PGPR-ISR against bacterial wilt and feeding by the cucumber beetle vectors of *E. trachiphiela* were associated with reduced concentrations of cucurbitacin, a secondary plant metabolite and powerful beetle feeding stimulant. In other experiments, treatment with PGPR led to ISR against bacterial wilt in the absence of the beetle vectors, suggesting that PGPR-ISR protects cucumber against bacterial wilt not only by reducing beetle feeding and transmission of the pathogen, but also through the induction of other plant defense mechanisms after the pathogen has been introduced into the plant. Additional greenhouse and field experiments are described in which PGPR strains were selected for ISR against cucumber mosaic virus (CMV) and tomato mottle virus (ToMoV). Although results varied from year to year, field-grown tomatoes treated with PGPR demonstrated a reduction in the development of disease symptoms, and often a reduction in the incidence of viral infection and an increase in tomato yield. Recent efforts on commercial development of PGPR are described in which biological preparations containing industrial formulated spores of PGPR plus chitosan were formulated and evaluated for use in a transplant soil mix system for developing plants that can withstand disease attack after transplanting in the field.

Abbreviations: PGPR – plant growth-promoting rhizobacteria, ISR – induced systemic resistance, CMV – cucumber mosaic virus, ToMoV – tomato mottle virus.

Introduction

The rhizosphere of plants is a zone of intense microbial activity, and some bacteria from this zone, termed rhizobacteria, exhibit active root colonization in the presence of the existing native microflora. Rhizobacteria that exert beneficial effects on plant development are referred to as plant growth-promoting rhizobacteria (PGPR) (Kloepper et al., 1980), because their application is often associated with increased rates of plant growth. Efforts to enhance the beneficial effects of PGPR by supplemental application to crops or soil were first reported in the 1950s from studies in the former Soviet Union, and later in Western countries (reviewed in Backman et al., 1997). PGPR were initially applied to enhance crop fertility by increasing the amount of available nitrogen (Cooper, 1959). Later, they were used as biological control agents for suppression of soilborne pathogens (Dunleavy, 1955; Broadbent et al., 1971, Schippers et al., 1987; Kloepper, 1993). PGPR antagonize soil pathogens by competing for resources such as iron, or by the production of antibiotics or lytic

enzymes (van Loon et al., 1998). In 1985, Gustafson, Inc. (Plano, Texas) introduced the first commercial rhizobacteria biological control products in the U.S. using the *Bacillis subtilis* A-13 strain (Broadbent et al., 1977) and related strains GBO3 and GBO7 (sold under the trade names Quantum®, Kodiak®, and Epic®, respectively). These products are used in combination with seed treatment fungicides to protect seed against attack by fungal soil pathogens. In China, PGPR have been in commercial development for over 20 years and are referred to as 'yield-increasing bacteria' (YIB) that are applied to over 20 million ha of crops (Chen et al., 1996).

During the 1980s, work on the mode of action of PGPR with biological control activity began to suggest that some PGPR strains may activate host defense systems based on lack of direct antibiosis of the strains toward pathogens or on correlation of biocontrol with plant growth promotion (Scheffer, 1983; Voisard et al., 1989). In 1991, direct evidence supporting the conclusion that PGPR which remain on plant roots can induce resistance in plants to foliar or systemic pathogens was published independently for three pathosystems: cucumber and anthracnose (Wei et al., 1991); carnation and Fusarium wilt (van Peer et al., 1991); bean and halo blight (Alström, 1991). Systemic resistance induced by PGPR has been termed 'induced systemic resistance' (ISR) (Kloepper et al., 1992; Pieterse et al., 1996). ISR is dependent on colonization of the root system by sufficient numbers of PGPR, and this has been achieved by coating seed with high numbers of bacteria or by adding bacterial suspensions to soil before sowing or at transplanting (Kloepper, 1996). Studies to elucidate the plant biochemical pathways associated with induction by PGPR were reviewed by van Loon et al. (1998).

The first successful field trials with PGPR were conducted in cucumber and demonstrated that seed treatment followed by soil drench application resulted in a reduction of bacterial wilt disease symptoms (Wei et al., 1995) and also the control of bacterial angular leaf spot and anthracnose (Wei et al., 1996). Encouraged by these results, we initiated experiments to investigate the factors involved in PGPR-mediated resistance to the bacterial wilt pathogen in cucumber. In this article results from these experiments will be reviewed and two additional projects to identify PGPR strains that could induce resistance to insect transmitted virus diseases in field-grown tomato will be described. Recent efforts to develop biological preparations containing industrial formulated spores of PGPR for growth promotion and disease control in vegetable transplant production systems will also be reviewed.

Resistance against cucumber beetles and bacterial wilt disease

Bacterial wilt of cucurbits is a systemic disease caused by the xylem-inhabiting bacterial pathogen *Erwinia tracheiphila* (Smith), which survives in, and is transmitted by the spotted (*Diabrotica undecimpunctata* howardi Barber) and striped (*Acalymma vittata* [Fabricius]) cucumber beetles. *E. tracheiphila* is thought to be entirely dependent on cucumber beetles for inoculation and dissemination in the field (Agrios, 1978), and there is a direct relationship between beetle density and severity of disease (Yao et al., 1996). The primary control method for bacterial wilt involves use of synthetic insecticides targeted against cucumber beetle. Cucumber beetle feeding behavior and damage is strongly influenced by cucurbitacins, a group of triterpenoid plant metabolites that occur in the plant family Cucurbitaceae (Chambliss and Jones, 1966). Cucurbitacins act as powerful feeding stimulants for cucumber beetles (Chambliss and Jones, 1966; Metcalf, 1986), and even very low concentrations (i.e., 0.001μg) stimulate compulsive feeding behavior (Metcalf, 1986).

We first suspected that cucumber beetle feeding behavior was affected by PGPR treatment following cucumber field experiments in which PGPR afforded unexpected protection against bacterial wilt disease with large numbers of cucumber beetles present (Wei et al., 1995). Field studies were then initiated to assess the effects of PGPR treatment on field populations of cucumber beetles, and to compare PGPR treatment with weekly applications of insecticide for cucumber beetle control (Zehnder et al., 1997a). Greenhouse and laboratory experiments were also conducted to determine if resistance against feeding by cucumber beetles was a factor in PGPR-induced protection against bacterial wilt that was previously observed in the field, and to quantify cucurbitacin content in PGPR-treated and nontreated cucumber (Zehnder et al., 1997b).

Field experiments

Field studies were conducted in 1993 and 1994 to assess the effects of PGPR treatment on populations of cucumber beetles, and to compare PGPR treatment

with weekly applications of insecticide for control of cucumber beetles and bacterial wilt disease on cucumber (Zehnder et al., 1997a). For these experiments, PGPR strains that were shown previously to reduce bacterial wilt disease incidence in cucumber were used (Wei et al., 1995). Cucumber seeds were dipped in pelleted bacterial cells or into distilled water (control) immediately before planting in plastic pots containing sterilized soilless planting mix. A dilute PGPR suspension (100 ml containing $\sim 10^8$cfu/ml) was poured into each pot immediately after seeding. Seedlings (cv Straight 8) were transplanted into the field at the 2nd true leaf stage and grown in fumigated (methyl bromide + chloropicrin), raised beds with black plastic mulch and drip irrigation. Treatments in 1993 included the following PGPR: *Pseudomonas putida* strain 89B-61, *Serratia marcesens* strain 90-166, *Flavomonas oryzihabitans* strain INR-5, and *Bacillus pumilis* strain INR-7. Control treatments included an insecticide control (weekly sprays of esfenvalerate by backpack sprayer) and a nontreated control. The 90-166 and INR-7 strains were re-evaluated in 1994 along with the insecticide and nontreated controls.

Average counts of cucumber beetles in both years of the study were significantly lower in the PGPR treatments compared with the nontreated control (Table 1). In the second year, when bacterial wilt disease symptoms were observed, the average percentage of wilted vines was significantly lower in the PGPR treatments than in the nontreated control. In both years, yields in the PGPR treatments were significantly higher than in the nontreated controls. It is interesting to note that beetle counts and wilt symptoms in some PGPR treatments were significantly lower than in the insecticide treatments. These results indicate that, given our experimental conditions, the PGPR treatments were more effective than the insecticide treatment.

Table 1. Comparison of PGPR and control treatments for control of cucumber beetles and bacterial wilt disease in cucumber field experiments

PGPR treatment	Mean no. beetles/plant		Mean % wilted vines	Mean fruit weight/plot (kg)	
	1993	1994	(1994)	1993	1994
89B61	0.61 cd	NT	NT	37.3 a	NT
90-166	0.44 d	2.34 c	2.61 c	35.9 a	28.1 a
INR-5	0.56 cd	NT	NT	32.7 ab	NT
INR-7	0.73 bc	2.96 bc	3.35 bc	37.1 ab	26.5 ab
Insecticide Control*	0.89 b	NT	11.48 b	25.6 b	21.9 ab
Nontreated	1.73 a	5.42 a	24.56 a	29.4 b	20.8 bc

NT, not tested. Means within columns sharing a letter in common are not significantly different ($P > 0.05$; LSD test). Beetle and wilted vine means derived from 6 replicates; 10 plants per replicate. Beetle data averaged over 6 sample dates; wilted vines recorded on 24 June, 1994.

*Plants sprayed weekly with esfenvalerate insecticide at the rate of 0.05 lb (AI)/acre.

The aforementioned studies used standard vegetable production practices which include preplant soil fumigation with methyl bromide. Additional field studies evaluated growth promotion and PGPR-ISR in cucumber plots with and without methyl bromide soil fumigation (Zehnder et al., 2000a). In both fumigated and nonfumigated plots, numbers of cucumber beetles and the incidence of bacterial wilt disease were significantly lower with PGPR treatment compared with the nonbacterized control. However, in PGPR-treated plots, the incidence of bacterial wilt was significantly lower in the nonfumigated treatments compared with fumigated treatments, indicating that the level of PGPR-mediated ISR was greater without methyl bromide fumigation. This suggests that soil fumigation had a negative effect on PGPR-ISR, possibly by elimination of symbiotic soil microfauna. Plant height measurements demonstrated that cucumber plant growth rates in nonfumigated PGPR treatments were equivalent to growth rates in the fumigated treatments without PGPR. These results indicate that in cucumber production systems, PGPR may have potential as a biological alternative to methyl bromide fumigation.

Greenhouse and laboratory experiments

Greenhouse and laboratory experiments assessed whether the observed PGPR-induced protection against bacterial wilt resulted from ISR against the pathogen, the vector, or both. In free-choice experiments, screen cages designed in a 'cross' arrangement with 4 arms (see Zehnder et al., 1997b for complete details) were used to confine cucumber beetles on PGPR-treated (seed treatment and transplant drench with INR-7 strain) or nontreated plants. PGPR-treated plants were placed in 2 arms/cage, and nontreated plants in the other 2 arms/cage; 2 cages were used for each experiment (4 treatment replicates per experiment, 8 plants per replicate). Experiments were repeated twice. At the start of each experiment, 100 spotted cucumber beetles were

confined on *E. tracheiphila*-infected cucumber plants in the center 'cage within a cage' for 48 h before doors were opened allowing beetles free access to all 4 cage arms. Data on beetle feeding damage and wilt incidence were recorded at 13 (experiment 1) or 17 (experiment 2) d after beetle release on noninfected plants.

The beetles which were given a choice preferred nontreated to PGPR-treated plants, as evidenced by more feeding damage on stems and cotyledons and a higher incidence of bacterial wilt on the nontreated plants (Figure 1). Separate, no-choice experiments also demonstrated that spread of *E. tracheiphila* by cucumber beetles was significantly reduced by PGPR treatment, even when beetles were restricted to feeding only on PGPR-treated plants (Zehnder et al., 1997a).

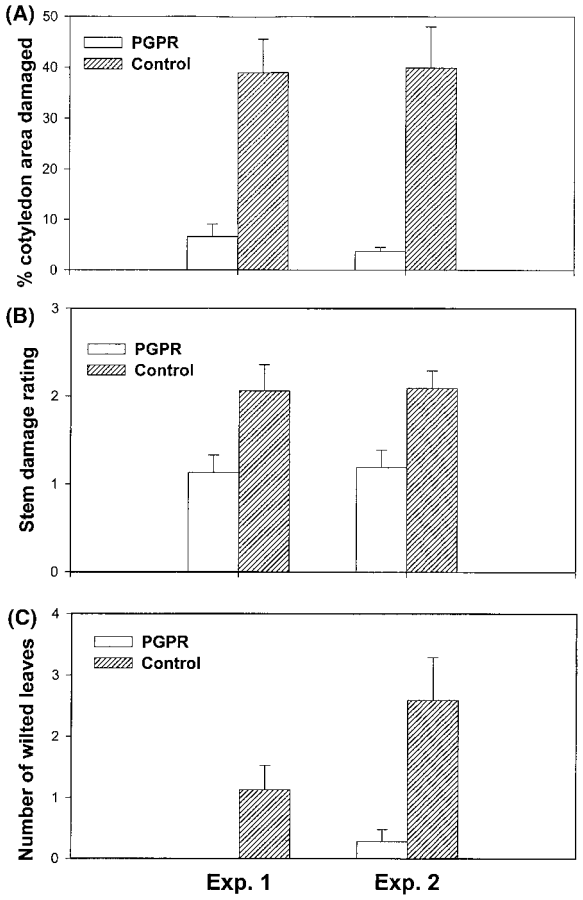

Figure 1. Cucumber beetle feeding damage and incidence of bacterial wilt symptoms on PGPR and untreated (control) cucumber plants. Free choice experiments were done in screen cages and repeated twice. (A) Mean percentage of cotyledon leaf area per plant with feeding damage. (B) Mean stem feeding damage rating per plant; 1 = <1/3 of stem from soil line to cotyledons damaged; 2 = 1/3 to 2/3 of stem damaged; 3 = >2/3 stem with feeding damage. (C) Mean number of wilted leaves per plant.

When cucumber beetles were released into cages containing both 'bitter' (*BI*, high cucurbitacin) and 'nonbitter'(*bi*, zero or low cucurbitacin) cucumber isolines, the beetles exhibited immediate preference for *BI* plants (Yao, 1995) (Figure 2). This demonstrated that the beetles discriminated between *BI* and *bi* plants and confirmed that cucurbitacin content alone can influence beetle feeding preference. To explain how treatment with PGPR could result in reduced beetle feeding on cucumber, we hypothesized that PGPR-treated plants accumulate lower concentrations of cucurbitacins. To test this, HPLC analysis was done to detect cucurbitacin 'C', the primary cucurbitacin in cucumber, in cotyledon leaf samples from PGPR-treated (INR-7 and INR-5 strains) or nontreated plants (see Zehnder et al., 1997b for analytical methods). HPLC analysis confirmed our hypothesis; significantly lower levels of cucurbitacin 'C' were detected in induced plants (Table 2). This occurred in both the Poinsett isoline

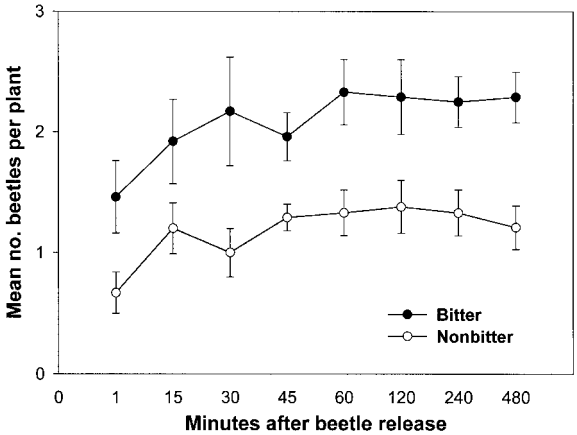

Figure 2. Mean number of cucumber beetles/plant over time after release in screen cages on 'bitter' (high cucurbitacin) and 'nonbitter' (zero cucurbitacin) cv 'Poinsett' cucumber isolines. Five each of bitter and nonbitter plants at the second true leaf stage of growth were placed on opposite ends of each of the 4 arms of the cage (Yao et al., 1995). At the start of the experiment, 25 spotted cucumber beetles in an open container were placed in the center of each arm equidistant from the bitter and nonbitter plants. The number of beetles per plant was determined 1 min after release and at 15 min intervals thereafter until 60 min after release, and at 2, 4 and 8 h. The experiment was replicated 4 times for each bitter vs nonbitter comparison.

with high cucurbitacin content, and in the Straight 8 cultivar with low cucurbitacin content.

To determine whether PGPR-mediated ISR to bacterial wilt is affected by cucurbitacin in the absence of the cucumber beetle vector, a greenhouse experiment was conducted in which *BI* and *bi* cucumber plants, either treated or nontreated with PGPR, were injected with extracts from *E. tracheiphila*-infected stems (Yao, 1995). PGPR significantly protected both *BI* and *bi* plants from wilt disease development (Table 3). Factorial analysis of variance indicated no significant bitterness × PGPR interaction, suggesting that cucurbitacin content does not influence PGPR-mediated ISR in the absence of the vector.

Discussion

Although not yet confirmed, the observed PGPR-mediated effects on cucurbitacin production may be the result of a shift in the metabolic pathway in which an increase in the production of plant defense compounds may result in deficiencies in other compounds requiring the same chemical precursors or intermediates. As demonstrated here, PGPR which induce systemic resistance inhibited accumulation of the secondary plant metabolite cucurbitacin. This could occur by a shift in the metabolic pathway to produce other anti-microbial compounds, as was observed in potato where fatty acids from *Phytophthora infestans* elicited the accumulation of sesquiterpenoid phytoalexins (Tjamos and Kuć, 1982). This increase was associated with a shift in the terpenoid pathway leading to reduced production of steroid glycoalkaloids that are used by the fungus. Since cucurbitacin and known defense compounds are synthesized from similar precursors (Balliano et al., 1982), a facultative alteration in metabolic pathway is a probable mechanism for the reduction in cucurbitacin observed in cucumber with ISR.

Collectively, these results suggest that PGPR-mediated ISR protects cucumber against bacterial wilt at two levels. First, the reduction in cucurbitacin synthesis in PGPR-treated plants makes these plants less palatable to cucumber beetles, which may result in a lower proportion of beetles acquiring and successfully transmiting the pathogen. Second, PGPR may elicit the induction of other plant defense mechanisms (i.e., phytoalexin production and other compounds involved in ISR) against the pathogen after it has been introduced into the plant.

Table 2. Effect of PGPR treatment on cucurbitacin 'C' concentration in 'Poinsett' ('bitter') and 'Straight 8' cucumber

PGPR treatment	Mean cucurbitacin concentration (μg/g)*	
	Poinsett	Straight 8
INR-7	117.3 b	27.1 c
INR-5	117.0 b	35.2 bc
Nontreated	158.6 a	48.4 a
LSD, $\alpha = 0.05$	27.3	9.6

Means within columns sharing a letter in common are not significantly different ($\alpha = 0.05$; LSD test).
*Cucurbitacin 'C' values are μg cucurbitacin/g fresh weight plant material. Means derived from 5 replicates per treatment (4 cotyledons from 2 plants per replicate).

Induced resistance against cucumber mosaic virus

Plant diseases caused by insect-transmitted viruses are among the most serious production problems encountered by vegetable growers. Effective insecticidal control of insect-borne virus diseases is problematic

Table 3. Comparison of PGPR-induced resistance to bacterial wilt infection in 'Poinsett' bitter (*BI*) and nonbitter (*bi*) cucumber isolines

Days after inoculation	Mean % wilted leaves/plant				ANOVA statistics					
	Bitter		Nonbitter		PGPR treatment		Cucumber isoline		Interaction	
	90–166	Control	90–166	Control	*F*	*P*	*F*	*P*	*F*	*P*
4	4.43	5.10	4.33	3.63	0.00	0.96	4.81	0.03	3.66	0.06
7	19.50	30.67	19.67	28.5	41.89	0.0001	0.42	0.52	0.57	0.45
10	69.17	91.80	71.83	94.0	118.38	0.0001	1.82	0.18	0.01	0.94

Each PGPR (PGPR or nontreated) and isoline (*BI* or *bi*) treatment consisted of 5 plants; treatments were replicated 6 times. The percentage of wilted leaves/plant was determined at 4, 7 and 10 days after inoculation. Percentage data were transformed before analysis by converting the square root of each proportion to arcsin; data were analyzed using a two-way factorial analysis of variance (ANOVA).

because most vectors are highly mobile insects and may colonize fields rapidly before growers are aware of their presence. In addition, viruses transmitted by aphids in a nonpersistent manner may be acquired during brief probes on infected plants, and healthy plants may in turn be inoculated too quickly for insecticides to have an effect. Cucumber mosaic cucumovirus (CMV) is one of the most important viruses affecting vegetables worldwide (Sherf and McNab, 1986; Tomlinson, 1987). CMV is difficult to control because of its extremely broad natural host range in excess of 800 plant species, and the ability to be transmitted in a nonpersistent manner by more than 60 species of aphids (Zitter, 1991; Palukaitis et al., 1992). In Alabama, insecticides were completely ineffective in preventing a serious epidemic of CMV on tomato (Sikora et al., 1998). CMV epidemics have also been reported in tomato-growing regions of Italy (Kaper et al., 1990), Spain (Jorda et al., 1992) and China (Kearny et al., 1990). There are no sources of genetic resistance to CMV available in commercial fresh market tomato cultivars (Sikora et al., 1998).

In greenhouse studies, Raupach et al. (1996) showed that two PGPR strains, which previously induced resistance in cucumber against fungal and bacterial diseases, also induced resistance to CMV in cucumber and tomato. Our study was done to screen additional PGPR strains for activity against CMV on greenhouse-grown tomato, and to determine if PGPR-mediated induced resistance could be extended to tomato grown in the field using commercial production practices.

Greenhouse experiments

Greenhouse experiments were first done to evaluate 26 PGPR strains for induced resistance against CMV in tomato. The PGPR strains were tested along with a disease control (CMV mechanical inoculation, no PGPR) and a healthy control (no CMV inoculation, no PGPR) (see Zehnder et al., 2000b for complete details). PGPR were applied to seed as pelleted bacterial cells at a density of approximately 5×10^9 cfu/seed. After transplanting into plastic pots, PGPR suspension treatments (100 ml containing approximately 5×10^8 cfu/ml) were poured into each pot immediately after transplanting. A water/buffer solution was applied to control plants. A CMV isolate collected from tomato in North Alabama was maintained in tobacco and used to inoculate plants (carborundum dusting followed by rub inoculation) in the PGPR and disease control treatments. Plants were examined daily for CMV symptoms (leaf distortion, mosaic patterns, general stunting of the plant). Based on results of two initial screening experiments, 16 of the most effective PGPR strains were evaluated in a third experiment, and the 8 strains exhibiting the highest level of protection were tested again in two additional trials (experiments 4 and 5). The number of plants exhibiting CMV symptoms was reduced in several PGPR treatments, compared with the disease control (Table 4). The percentage of plants showing symptoms in these PGPR treatments ranged from 32% to 58%, compared with 88–98% in the disease control treatment. Based on these results, 4 strains were chosen for further evaluation in field experiments.

Table 4. Results of greenhouse experiments to identify effective PGPR strains for induced resistance against cucumber mosaic cucumovirus (CMV) on tomato

PGPR strain or treatment	Mean no. symptomatic plants ± SEM[1]	
	4th experiment	5th experiment
BE55	3.5 ± 1.3	6.0 ± 1.4
IN266	4.5 ± 2.1	7.0 ± 0.8
SE34[2]	4.0 ± 1.4	5.8 ± 1.7
IN937a[2]	4.2 ± 1.2	5.0 ± 2.1
IN937b[2]	3.2 ± 1.0	4.8 ± 1.7
TE5	4.0 ± 1.6	6.5 ± 1.3
IN114[2]	3.5 ± 1.3	5.8 ± 1.7
89B-27	4.5 ± 2.1	6.8 ± 1.0
Nonbacterized, challenged control[3]	8.8 ± 1.0	9.8 ± 0.5
Nonbacterized, unchallenged control[4]	0	0

[1]Means calculated based on 40 plants per treatment/experiment.
[2]Selected for further evaluation in field trials.
[3]Plants inoculated with CMV and not treated with PGPR.
[4]Plants not inoculated with CMV and not treated with PGPR.

Field experiments

Field experiments were done in 1996 and 1997 to evaluate 4 PGPR strains, a disease control and a healthy control for induced resistance against CMV (see Zehnder et al., 2000b for complete details). The PGPR strains chosen for evaluation were *Bacillus pumilus* strain SE34, *Kluyvera cryocrescens* strain IN114, *Bacillus amyloliquefaciens* strain IN937a, and *Bacillus subtilus* strain IN937b. PGPR were applied to seed and as a transplant drench as was done in greenhouse experiments. Before transplanting in the field, tomato

plants in the PGPR and disease control treatments were mechanically inoculated with CMV as described above. There were 6 replications per treatment arranged in a randomized block design, each consisting of 15 tomato plants (single row plots). Tomato plants were grown on raised beds with drip irrigation, fumigated with methyl bromide/chloropicrin and covered with black plastic mulch (according to local tomato growing practices). All plants in each treatment were examined weekly for virus symptoms using a rating scale from 0 to 10, followed by a calculation of disease severity (Zehnder et al., 2000b). Marketable (nondamaged and mature) tomato fruit were weighed on 6 harvest dates during the season.

In 1996, ELISA values in all PGPR treatments, and the percentage of infected plants (based on ELISA) in 3 PGPR treatments, were significantly lower than in the disease control (Table 5). The percentage of infected plants in the disease control treatment was over 3-fold greater than the IN937a and IN937b treatments. Importantly, yields in the SE34, IN937a and IN937b treatments were significantly greater than in the disease control.

Overall, the percentage of plants infected with CMV was higher in 1997 than in 1996. In 1997, 62.2% of the nonchallenged, 'healthy' control plants tested positive for CMV by ELISA, compared with 4.4% in 1996. ELISA absorbance values in 1997 were significantly lower in the PGPR treatments than in the disease control, but the percentages of infected plants were not significantly different among treatments. Tomato yields overall were lower in 1997 than 1996, and not significantly different among treatments.

Discussion

These results provide evidence that PGPR-ISR against CMV on tomato, previously reported from greenhouse experiments (Raupach et al., 1996) and confirmed here, can be obtained under field conditions. However, the level of PGPR-induced resistance in the field was variable. In the 1996 experiment, the incidence of CMV infection was significantly reduced on PGPR-treated plants that were mechanically challenged with virus before transplanting in the field. In addition, tomato yields from PGPR-treated plants were not significantly different from yields on healthy control (nonbacterized, unchallenged) plants. Although ELISA values in 1997 were significantly lower in PGPR treatments than in the healthy control, the significant effects of PGPR on the incidence of infected plants and on tomato yields, as seen in 1996, were not evident. In 1997, a much greater proportion of unchallenged control plants tested positive for CMV than in 1996. A possible explanation for the greater incidence of infection in 1997 is that the plants were subjected to higher levels of naturally transmitted CMV than in 1996 (e.g., aphids migrating into the area spread CMV from plant to plant thereby supplementing levels of CMV in mechanically challenged plants). Consequently, in 1997, PGPR-induced plant defense mechanisms may have been unable to compensate for the greater viral load. Another explanation for reduced effectiveness of PGPR in 1997 could be that plants were naturally infected with a different strain of CMV, and that the PGPR strains tested were not as effective against the naturally occurring CMV strain. These results show that the level of protection resulting

Table 5. Effects of PGPR treatment on cucumber mosaic virus (CMV) infection and yield in tomato field plots, 1996 and 1997

Treatment	ELISA value		% Plants infected based on ELISA		Average yield (kg/plot)	
	1996	1997	1996	1997	1996	1997
SE34	0.18 c	0.27 b	30.0 b	64.4 a	14.0 a	3.2 a
IN114	0.30 b	0.29 b	58.8 a	68.9 a	10.3 b	2.4 a
IN937a	0.12 cd	0.26 b	21.1 b	55.8 a	14.8 a	2.5 a
IN937b	0.12 cd	0.25 b	17.7 b	65.6 a	14.2 a	2.1 a
Nonbacterized, challenged control	0.48 a	0.37 a	66.7 a	83.3 a	9.5 b	2.0 a
Nonbacterized, unchallenged control	0.05 d	0.26 b	4.4 c	62.2 a	14.1 a	2.9 a
$LSD_{0.05}$	0.09	0.07	13.4	27.9	2.4	1.54

Means within columns sharing the same letters are not significantly different ($P > 0.05$; LSD test).

from treatment by a given PGPR strain may vary from one cropping season to the next depending on existing conditions. We have not yet conducted experiments specifically to evaluate PGPR on tomato for induced resistance against CMV by natural aphid transmission, or to measure the effects of changing abiotic factors on PGPR-induced resistance.

Induced resistance against tomato mottle virus

Tomato mottle virus (ToMoV) has been a major yield-limiting factor in Florida tomato production since the early 1990s (Kring et al., 1991; McGovern et al., 1995; Simone et al., 1990). ToMoV is transmitted by adult sweet potato whiteflies, *Bemisia tabaci* [Gennadius], biotype B (also known as the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring). Similar to the CMV pathosystem, management of ToMoV has been difficult because genetically resistant tomato varieties are not available, and because of the ability of whiteflies to develop resistance to insecticides (Denholm et al., 1996; Stansly et al., 1991). Prompted by our findings that PGPR-ISR resulted in protection against CMV on tomato, field trials were conducted to evaluate some of the same PGPR strains for induced resistance in tomato against ToMoV.

Field experiments

Experiments were conducted in Bradenton, Florida, USA during the fall tomato growing season in 1997, and during the spring and fall growing seasons in 1998 (Murphy et al., 2000). PGPR strains were chosen on the basis of their effectiveness for induced resistance against CMV on tomato. In 1997, PGPR strains IN937b and IN937a were evaluated, and these two strains plus strain SE34 were evaluated in 1998. Spore preparations of each PGPR strain were produced in culture and formulated as a seed treatment and as a powder by Gustafson, Inc. (Plano, Texas). The powder treatments were diluted with water according to the manufacturer's recommendations and incorporated into the planting mix before seeding.

Tomato cv. Agriset transplants were set into raised beds covered with either black (spring experiment) or white (fall experiments) polyethylene plastic film. Beds were fumigated with methyl bromide before application of plastic. Single row treatment plots were replicated four times in a completely randomized design and consisted of 10–12 plants per row. Tomatoes were inoculated with ToMoV by natural movement of viruliferous whiteflies from adjacent, infected tomato germplasm being developed for ToMoV resistance. Each tomato plant was evaluated for disease severity at 40 days after planting using a scale of 0–5.0 (Murphy et al., 2000). In addition to the symptom rating, leaflet samples from each plant were tested for the presence of ToMoV DNA by Southern dot-blot analysis (Polston et al., 1993). The total weight of all undamaged, mature tomato fruit was recorded from each plot at least 3 times during each season.

In the 1997 experiment, ToMoV symptom ratings in the IN937a and IN937b powder and seed + powder formulation treatments were significantly lower than in the control (Table 6), but ratings in the seed treatment formulations did not differ from the control. Results of the Southern dot-blot analysis corresponded with the symptom ratings; e.g., the percentage of tomato plants infected by ToMoV was lower in all powder-based treatments compared to plants subjected to seed treatment alone or the control treatment. Data on fruit weight from the first harvest indicated that plants in each of the powder-based PGPR treatments produced higher yields than plants in the control treatment. First harvest yields in the PGPR seed treatments were not significantly different from the control. Overall yields

Table 6. Response of field tomato subjected to different PGPR treatments and formulations to infection by ToMoV, Fall 1997

PGPR strain/formulation	ToMoV symptom rating[1]	% of plants testing positive for ToMoV by dot-blot assay	Yield (kg/plot)[2]
IN937a seed	2.80 a	70 a	46.3 b
IN937b seed	2.66 a	65 a	49.2 ab
IN937a powder	1.42 b	28 ab	55.2 ab
IN937b powder	1.52 b	21 b	68.4 a
IN937a seed+ powder	1.12 b	13 b	55.8 ab
IN937b seed+ powder	1.60 b	30 ab	49.4 ab
Untreated control	2.64 a	55 ab	47.0 b

Means within a column with the same letter are not significantly different ($P > 0.05$; LSD test).

[1]Ratings based on a 5 point scale: 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; 5 = obvious mottling on leaves, leaf distortion an severe stunting.

[2]Total marketable yield of undamaged, mature fruit on the first harvest date.

were lower in the second and third harvests, with no consistent differences among treatments (data not shown).

In the spring 1998 experiment, PGPR SE34 strain was used in place of IN937a with the same application methods as in the 1997 trial. Compared with the control, ToMoV symptom ratings were significantly lower in the IN937b seed, SE34 powder and SE34 seed + powder treatments. (Table 7). The percentage of plants testing positive for ToMoV DNA was significantly lower in the IN937b seed and SE34 seed + powder treatments. Similar to trends shown by the symptom rating data, the percentage of ToMoV positive plants was significantly lower in the SE34 seed + powder treatment than in the SE34 seed treatment. Overall fruit yields were lower in the spring 1998 experiment than in the 1997 trial. First harvest yields were highest in the IN937b seed + powder treatment, the only PGPR treatment with significantly greater yields than the control. No differences in yield were observed among treatments from the second and third harvests (data not shown).

In the fall 1998 experiment (data not shown), there were no significant differences in ToMoV disease severity ratings or in the percentage of plants testing positive for ToMoV DNA between the PGPR treatments and the control. Similarly, fruit yields from plants in the PGPR treatments did not differ significantly from those in the control.

Table 7. Response of field tomato subjected to different PGPR treatments and formulations to infection by ToMoV, Spring 1998

PGPR strain/ formulation	ToMoV symptom rating	% of plants testing positive for ToMoV by dot-blot assay	Yield (kg/plot)[1]
SE34 seed	3.57 a	90 ab	15.8 bc
IN937b seed	2.60 b	65 bc	9.8 c
SE34 powder	2.57 b	75 a–c	11.8 bc
IN937b powder	3.00 ab	70 a–c	17.8 ab
SE34 seed + powder	2.40 b	55 c	13.5 bc
IN937b seed + powder	2.83 ab	73 a–c	22.8 a
Untreated control	3.45 a	93 a	12.8 bc

Means within a column with the same letter are not significantly different ($P > 0.05$; LSD test).

[1]Ratings based on a 5 point scale: 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; 5 = obvious mottling on leaves, leaf distortion an severe stunting.

[2]Total marketable yield of undamaged, mature fruit on the first harvest date.

Discussion

These results demonstrate that treatment of tomatoes with PGPR can provide protection in the field against ToMoV under natural conditions. Although the observed level of ToMoV control varied among experiments, the results were encouraging given that the PGPR strains used in the Florida trial were selected based on screening for CMV (in the cucumovirus family) and not ToMoV (a geminivirus). This illustrates the potential of PGPR to provide protection against multiple pathogens. Although protection against ToMoV was observed, there was no consistent trend in resistance induced by any particular PGPR strain or formulation from one trial to another. The greatest protection occurred in the fall 1997 trial where all of the powder-based PGPR treatments resulted in reduced disease severity and incidence of ToMoV.

It is interesting to note that, in the fall 1997 trial, numbers of whitefly nymphs were significantly lower on plants in four of the PGPR treatments compared with the control (Murphy et al., 2000). Of the four treatments, three included the IN937a and IN937b powder formulations that also resulted in reduced ToMoV disease severity. Similarly, in the spring 1998 trial, whitefly densities were reduced in four of the PGPR treatments. Although not directly tested in these experiments, the results suggest that PGPR-ISR may effect whitefly host preference or development. It is not known whether reduced whitefly densities on PGPR-treated tomato resulted in a lower dosage of pathogen introduced into the plant, as was observed with cucumber beetles and bacterial wilt disease (Zehnder et al., 1997b). Mechanical inoculation was used in our studies with PGPR-ISR against CMV, indicating that the resistance was effective against some stage in the CMV infection process rather than interference in transmission. Additional experiments are needed to determine if the observed resistance against ToMoV resulted from defense against the pathogen, the vector, or both.

Commercial potential of PGPR

Increasing public concern for the environment has resulted in more stringent government controls over

pesticide registration, as evidenced by the Food Quality Protection Act passed into law by the U.S. Congress in 1996. This has created a need for rapid development and implementation of effective biological products for pest management. Therefore, it is likely that the market for commercial PGPR products will continue to expand. Backman et al. (1997) reported that 60–75% of the U.S. cotton crop is treated with Kodiak®, the *B. subtilis* product used for suppression of *Fusarium* and *Rhizoctonia* soil pathogens. Kodiak® is also used in peanut, soybean, small grain, corn and vegetable crops. In China, 18 commercial PGPR strains or strain mixtures are sold, most of which are derived from the spore-forming genus *Bacillus* (Backman et al., 1997).

PGPR are ideal vehicles in which to deliver crop protection because they can be applied to seed or mixed with soil at seeding or transplanting. In addition to direct control of soil pathogens, the studies reviewed in this article demonstrate that PGPR represent an attractive alternative to chemical pesticides for systemic protection against foliar pathogens. A major advantage of PGPR is that once systemic resistance is induced, the natural defense mechanisms of the plant are operative for prolonged periods even if populations of inducing bacteria decline over time (van Loon et al., 1998). Researchers at Auburn University and Gustafson, Inc. are working on the next generation of PGPR products that will provide growth promotion and systemic disease protection in addition to protection against soil pathogens. These products are formulated for use in a transplant soil mix system for developing 'suppressive plants' which can withstand various pests upon transplanting into agricultural fields. The mix contains a combination of PGPR strains that are selected based on the crop and pest system, plus chitin that is added as a formulation carrier. Recent studies have shown that amendment of soil with chitin containing amendments enhances general soil suppressiveness to soil pathogens and nematodes through alterations in microbial community structure (Kloepper et al., 1999). In addition, the addition of chitosan with PGPR appears to synergize plant growth promotion and ISR activity (Reddy et al., 1999). One product currently in development, called LS213, contains industrial formulated spores of *B. subtilis* strain GBO3, *B. amyloliquefaciens* strain IN937a, and chitosan as a formulation carrier. In recent greenhouse trials, LS213 significantly increased growth of tomato, cucumber, tobacco and pepper transplants, and provided protection against bacterial spot and late blight of tomato, angular leaf spot of cucumber, and blue mold of tobacco (Reddy et al., 1999). Vegetables produced with LS213 exhibited significant protection against nematode damage and against anthracnose (cucumber) and bacterial spot (tomato) diseases after transplanting in the field (Kenney et al., 1999). PGPR amendments, including LS213, have also been used to enhance pine seedling root and shoot growth in the production of containerized forest-tree seedlings (Enebak et al., 1999).

It remains to be determined if these PGPR products used alone will consistently provide acceptable levels of disease control. As reported in this article, levels of disease protection afforded by PGPR vary from year to year depending on existing environmental conditions. Therefore, the best approach may be to combine PGPR with other pest management strategies, such as resistant or tolerant crop varieties, cultural practices, i.e., a reflective mulch to repel insect virus vectors, or other inducing agents that suppress diseases by complementary mechanisms, i.e., benzothiadiazole (Görlach et al., 1996; Tally et al., 1999). Furthermore, PGPR product development will be driven by economic considerations that may restrict its use to certain markets. Certainly, PGPR represent a potentially valuable crop protection tool in high value cropping systems like vegetables where regulations or lack of efficacy limit the availability of chemical crop protectants.

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