Induction of Systemic Resistance in Cucumber Against Cucumber Beetles (Coleoptera: Chrysomelidae) by Plant Growth-Promoting Rhizobacteria

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ABSTRACT Field studies were conducted in 1993 and 1994 to evaluate the effects of induced resistance in cucumber by plant growth-promoting rhizobacteria (PGPR) on numbers of the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barber, and the striped cucumber beetle, *Acalymma vittatum* (F.). Cucumber plant growth and yields were significantly ($P < 0.05$) greater, and populations of cucumber beetles were significantly lower, on PGPR-treated cucumber than on nontreated cucumber. On dates when peak beetle populations were present, PGPR treatment resulted in significantly ($P < 0.05$) greater cucumber beetle control than weekly applications of esfenvalerate insecticide. In no-choice greenhouse cage experiments with 3 cucumber cultivars, beetles infected with the cucurbit wilt pathogen, *Erwinia tracheiplila*, were released and allowed to feed on PGPR-treated or nontreated cucumber plants. The incidence of cucurbit wilt disease was significantly ($P < 0.05$) lower on PGPR-treated cucumber plants than on nontreated plants. These results indicate that PGPR-induced resistance may be more effective than insecticides for control of cucumber beetles and cucurbit wilt disease on cucumber. Possible mechanisms for PGPR-induced resistance against cucumber beetles are discussed.

KEY WORDS cucumber beetles, *Erwinia tracheiphila*, plant growth-promoting rhizobacteria, induced resistance

THE STRIPED CUCUMBER beetle, *Acalymma vittatum* (F.), and the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barber, are serious pests of cucurbits grown in the midwestern and eastern United States. Larvae feed on roots and stems, causing considerable damage, but the greatest direct injury results from adult feeding on cotyledons and stems of young plants (Brewer et al. 1987, Metcalf and Metcalf 1993, Foster et al. 1995).

Acalymma vittatum and *D. u. howardii* also are vectors of cucurbit wilt, a destructive vascular disease of cucurbits, caused by the bacterium *Erwinia tracheiphila* (Smith) (Bradbury 1970). Yield losses can be as high as 75%; cucumber and muskmelon are the most susceptible host crops (Sherf and MacNab 1986). Inoculation occurs through feeding wounds made by infected cucumber beetles, and evidence has been presented to support the theory that the pathogen overwinters in the beetle vectors (reviewed by Harrison et al.1980). Foster et al. (1995) indicate that nonsymptomatic weeds may serve as overwintering hosts for *E. tracheiphila* but evidence to support this hypothesis has not been published. Cucumber beetles usually colonize spring-planted cucurbits shortly after plant emergence (Hofmaster 1980, Yao et al. 1996). A positive, linear relationship has been demonstrated between cucumber beetle density on cucumber plants and the incidence of bacterial wilt symptoms (Yao et al. 1996).

The primary control for bacterial wilt involves use of insecticides targeted against the cucumber beetle vectors. The usual approach is the use of granular carbofuran (Furadan 15G [granular], FMC, Princeton, NJ) applied at planting as a systemic treatment for cucumber beetle control. Carbofuran, however, is a highly toxic pesticide (acute rat LD_{50} of 11 mg/kg) with high leaching capabilities (National Research Council 1989) and is prone to microbially enhanced degradation which limits its long-term usefulness (Buhler et al. 1992). Alternatively, growers can initiate a regime of weekly foliar insecticide applications; however foliar sprays are not greatly effective for preventing the spread of cucurbit wilt disease because cucumber beetles are highly mobile and exhibit great interplant and interfield movement. In addition, foliar insecticides may not be effective because of the low beetle population threshold for transmis-

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sion of the disease (Yao et al. 1996). A more desirable approach to cucumber beetle and cucumber disease management may be to focus on host plant resistance.

Induced resistance in plants has been the subject of considerable research over the past 2 decades with the discovery that many pathogens or chemical compounds may be used to elicit host defense mechanisms leading to reduced pathogen attack (Kuć 1983, 1987). Induced systemic resistance (ISR) is defined as systemic protection of a plant by an inducing agent when applied to a single part of the plant (Kloepper et al. 1992). Cucumber has been used as a model for induced resistance (Hammerschmidt et al. 1982; Kuć 1983, 1987; Métraux and Boller 1986), and cucumber resistance to bacterial wilt has been demonstrated by previous inoculation of lower leaves with fungal, bacterial, and viral pathogens (Bergstrom 1981).

More recently, plant growth-promoting rhizobacteria (PGPR) have been investigated as nonpathogenic agents or elicitors of ISR against various pathogens (Van Peer et al. 1991; Wei et al. 1991; Zhou and Paulitz 1994; Liu et al.1995a, b). PGPR are a subset of rhizosphere bacteria which colonize plant roots and exert beneficial effects on crop development, including plant growth promotion and biological disease control (reviewed by Kloepper et al. 1993). PGPR are ideal vehicles for delivering benefits to plants because they can be applied to seed directly with subsequent bacterial colonization of plant roots.

In field experiments to evaluate several PGPR strains for control of *Pseudomonas syringae* pv. *lachrymans*, causal agent of cucumber angular leaf spot, it was unexpectedly observed that PGPR afforded protection against bacterial wilt disease associated with the presence of large numbers of cucumber beetles (Wei et al. 1995). We hypothesized that reduced numbers of cucumber beetles on PGPR-treated plants may have been a factor in the observed protection against bacterial wilt. Here, we present results of field studies done to assess the effects of PGPR treatment on field populations of cucumber beetles on cucumber, and to compare PGPR treatment with weekly applications of insecticide for cucumber beetle control. We also report results of greenhouse cage experiments to assess the effect of PGPR treatment on transmission of *E. tracheiphila* by cucumber beetles.

Materials and Methods

PGPR Cultures and Seed Treatment. PGPR strains for evaluation were obtained from the Department of Plant Pathology, Auburn University, and were previously shown to reduce disease incidence in cucumber caused by *E. tracheiphila*. These were *P. putida* strain 89B-61, *Serratia marcescens* strain 90-166, *Flavomonas oryzihabitans* strain INR-5, and *Bacillus pumilus* strain INR-7. Bacteria were identified using fatty acid analysis (Sasser 1990) and were maintained at −80°C in tryptic soy broth (TSB) with 20% glycerol. For bioassay, cultures from storage were grown in tryptic soy agar and incubated for 24 h at 28°C. A loopfull of bacteria was then transferred to 1-liter flasks with TSB and shaken at 150 rpm (24°C) for 24 h. PGPR suspensions were centrifuged at 6,000 × g for 5 min. Cucumber seeds were dipped into the pelleted bacterial cells or into distilled water (control) immediately before planting in 10cm^2 plastic pots containing sterilized Promix soilless mix (Preauer Peat, Riviére-du-Loup, Québec, Canada). A dilute PGPR suspension (100 ml containing ≈10^8 colony-forming units/ml) was poured into each pot immediately after seeding. Seedlings were transplanted into the field at the 2nd leaf stage.

Field Experiments. 'Straight 8' cucumbers were transplanted into the field on 19 April 1993 and 15 April 1994 at the E. V. Smith Horticulture Substation, Shorter, AL. Cucumbers were grown in fumigated (335 kg/ha of 67% methyl bromide + 33% chloropicrin), raised beds with black plastic mulch and drip irrigation. Fertilization and weed control were done according to local cucumber production practices. Fungicides were not applied. Treatment plots consisted of 1 row (10 m long) with 0.9 m plant spacing. In 1993, a 7 treatment × 6 replication randomized complete block design was used. Treatments included PGPR strains 89B-61, 90-166, INR-5, and INR-7, an induced resistance (ISR) control (inoculation of cotyledons with *Colletotrichum orbiculare* 13 d before transplanting), an insecticide control (weekly sprays of esfenvalerate [Asana XL; Dupont, Wilmington, DE] at 56 g (AI)/ha by backpack sprayer delivering 374 liter/ha at 7.0 kg/cm^2 pressure), and a nontreated control. In 1994, a 4 treatment × 6 replication randomized complete block was used. Treatments included the strains INR-7 and 90-166, the insecticide control, and the nontreated control.

Beginning with initial colonization of plants by beetles, numbers of striped and spotted cucumber beetles were recorded weekly on 5 randomly chosen plants (foliage and flowers) per plot. Beetle samples were taken during the cooler mornings before 1000 hours to facilitate counting on plants before beetles became highly active. Plant growth was assessed 50 d after emergence by recording the length of the main runner and the number of leaves per plant on 5 randomly chosen plants per plot. Cucumbers were harvested at least twice weekly and weighed to determine fruit yield (cumulative fresh weight) in each plot. Data were analyzed using a single-factor analysis of variance (ANOVA), and treatment means were compared using the Newman–Keuls test (SAS Institute 1990).

Cage Experiments With Cucumber Beetles. Experiments were done in a controlled-temperature greenhouse with minimum (night) and maximum (day) temperatures of ≈24° and 28°C, re-

Table 1. Growth promotion in field cucumber resulting from PGPR treatment

Treatment	Mean main runner length/plant cm ± SEM, 1993	Mean leaf no./ plant ± SEM, 1993	Mean fruit wt/plot, kg ± SEM	
			1993	1994
89B-61	62.6 ± 1.5ab	29.5 ± 2.1ab	37.3 ± 2.7a	NT
90-166	64.9 ± 1.6a	30.1 ± 1.4a	35.9 ± 2.8a	28.1 ± 2.0a
INR-5	65.5 ± 2.3a	31.5 ± 1.8a	32.7 ± 3.8ab	NT
INR-7	65.6 ± 1.9a	30.3 ± 1.6a	37.1 ± 1.6a	26.5 ± 2.0ab
ISR control[a]	49.6 ± 2.9d	20.5 ± 1.8d	25.6 ± 1.9b	NT
Insecticide control[b]	58.8 ± 1.4bc	25.2 ± 0.8bc	29.4 ± 2.8ab	21.9 ± 2.1ab
Nontreated	55.9 ± 2.1c	25.0 ± 0.2c	27.3 ± 2.7b	20.8 ± 2.3bc

Means within columns followed by the same letter are not significantly different ($\alpha = 0.05$; Newman–Keuls test). Means derived from 6 replicates; 10 plants per replicate. Plant growth data taken 17 d after transplanting. NT, not tested.

[a] ISR, induced systemic resistance; plants in this treatment were inoculated with a conidial suspension of *Coletotrichum orbiculare* applied to cotyledons 13 d before transplanting.

[b] Plants sprayed weekly with esfenvalerate insecticide at the rate of 56 g (AI)/ha.

spectively, and natural (summer) daylight. Cucumber seeds (treated with PGPR strain 90-166 or nontreated) were planted in plastic pots as described previously and plants were introduced in screen cages at the 2nd–4th leaf stage.

This was designed as a no-choice experiment in which screen cages contained either PGPR-treated or nontreated plants. Screen mesh cages were 1.0 by 0.5 by 0.5 m with a top access door. Spotted cucumber beetles (2–4 d old, obtained from French Agricultural Research, Lamberton, MN) were released (25 per cage) and allowed to feed on 3 *E. tracheiphila*-infected cucumber plants (not treated with PGPR) placed in the center of each cage for 48 h before 5 healthy PGPR-treated or nontreated plants were introduced into each cage. *E. tracheiphila* source plants were infected by injecting 0.5 ml of stem extract from diseased cucumber tissue into the 2nd petiole of plants in the 2nd true leaf stage. The initial pathogen used to create disease was *E. tracheiphila* culture #NJH 1302 obtained from M. Havey, Department of Horticulture, University of Wisconsin, Madison.

An experiment was conducted separately for each of 3 cucumber cultivars. Each cage contained 1 replicate treatment (PGPR or nontreated control) and treatments were replicated 4 times. 'Poinset' bitter (BI; lot 88-298A) and nonbitter (Bi; lot 85-882) seed were obtained from the Department of Plant Breeding, Cornell University, Ithaca, NY). 'Straight 8' seed were obtained commercially. Wilt incidence was assessed by determining the percentage of wilted leaves per plant in each cage 17–23 days after introduction of infected beetles to test plants. The 3 plants per cage artificially infected with *E. tracheiphila* were not examined for wilt symptoms. Percentage data were analyzed using *t*-test analyses (SAS Institute 1990) after an arcsin transformation of the square root of each percentage (proportion).

Results and Discussion

Field Experiments. Plant growth promotion resulting from PGPR was demonstrated in the 1993 field experiments, where main runner length and leaf number per plant were significantly ($P < 0.05$) increased by all PGPR treatments compared with the ISR and nontreated controls (Table 1). Cucumber yield (fruit weight per plot) was significantly ($P < 0.05$) greater in most PGPR treatments compared with the ISR and nontreated controls in 1993 (Table 1). In 1994, yield in the 90-166 treatment was significantly (35%) greater than in the nontreated control and 30% greater than the insecticide control (not significant) (Table 1). Yields in the 1994 INR-7 treatment were 22–27% greater than in the insecticide and nontreated controls, respectively (Table 1), although differences were not significant ($P > 0.05$).

Numbers of striped and spotted cucumber beetles on foliage and flowers were combined in this study. Concurrent studies in adjacent experiment station plots indicated that beetles were more prevalent on foliage, and striped cucumber beetle was the predominant beetle species in both years (Yao et al. 1996). On 9 June, when peak beetle populations were recorded in 1993, beetle numbers were significantly ($P < 0.05$) lower in all PGPR treatment plots compared with the ISR and nontreated control plots (Table 2). In addition, beetle counts on this date in the PGPR treatment plots also were significantly ($P < 0.05$) lower than counts in the insecticide control where esfenvalerate was applied on a weekly basis. Beetle counts averaged over all sample dates were significantly ($P < 0.05$) lower in the PGPR treatments than in the ISR and nontreated control treatments.

The 90-166 and INR-7 strains were re-evaluated in 1994; 90-166 because it was associated with lowest beetle numbers in the field, and INR-7 because it offered good protection against cucurbit wilt (after artificial inoculation) in preliminary greenhouse experiments (data not shown). Although local beetle populations were >3 times greater in 1994 than in 1993, beetle numbers averaged over all sample dates in the 90-166 and INR-7 treatment plots were significantly ($P < 0.05$) lower than in the nontreated plots, and beetle counts in the 90-166

Table 2. Cucumber beetle counts in PGPR-treated field cucumber and in control treatments, 1993

Treatment	Mean no. beetles per plant ± SEM				
	26 May	2 June	9 June	15 June	Season avg
Nontreated	0.20 ± 0.08a	2.70 ± 0.38a	3.70 ± 0.30a	0.17 ± 0.07a	1.69 ± 0.18a
ISR control[a]	0 ± 0b	2.00 ± 0.25ab	2.77 ± 0.22b	0.20 ± 0.08a	1.24 ± 0.14b
Insecticide control[b]	0.07 ± 0.04b	1.40 ± 0.18bc	2.07 ± 0.17c	0.03 ± 0.03a	0.89 ± 0.10c
89B-61	0.03 ± 0.03b	1.23 ± 0.24bc	1.13 ± 0.20d	0 ± 0a	0.60 ± 0.09c
90-166	0 ± 0b	0.87 ± 0.16c	0.83 ± 0.14d	0.07 ± 0.04a	0.44 ± 0.06c
INR-5	0 ± 0b	1.07 ± 0.25bc	1.10 ± 0.20d	0.07 ± 0.04a	0.56 ± 0.09c
INR-7	0 ± 0b	1.50 ± 0.32bc	1.13 ± 0.18d	0.27 ± 0.11a	0.73 ± 0.11c

Means within columns followed by the same letter are not significantly different (α = 0.05; Newman–Keuls test). Beetle counts (spotted and striped cucumber beetle species combined) made from visual examination of the foliage and flowers on 5 plants per plot, 30 plants per treatment.

[a] ISR, induced systemic resistance; plants in this treatment were inoculated with a conidial suspension of *Coletotrichum orbiculare* applied to cotyledons 13 d before transplanting.

[b] Plants sprayed weekly with esfenvalerate insecticide at the rate of 56 g (AI)/ha.

plots were significantly ($P<0.05$) lower than beetle numbers in the insecticide control (Table 3). As in 1993, beetle counts in the PGPR treatments were significantly lower than in the insecticide control treatment on the date when peak numbers of beetles were present (25 May). These results indicate that, given our experimental conditions, the PGPR treatments were more effective than insecticides for control of cucumber beetle populations on cucumber.

Cage Experiments with Cucumber Beetles. Wilt symptoms were observed on test plants 5–9 d after their introduction into cages, demonstrating that beetles became infected with *E. tracheiphila* from the artificially inoculated plants, then successfully transmitted the pathogen to healthy test plants. The average wilt percentages in the 3 experiments ranged from 52.8 to 85.3% on the nontreated plants but only from 7.6 to 13.1% on the PGPR-treated plants (Fig. 1; PGPR and nontreated means within each cultivar significantly different at $P<0.05$). These results demonstrate that spread of *E. tracheiphila* by cucumber beetles on bitter and nonbitter cucumber cultivars is significantly reduced by PGPR treatment, even when beetles are restricted to feeding only on PGPR-treated plants. Therefore, the mitigating effect of ISR on the spread of cucurbit wilt by cucumber beetles does not appear to be diminished by prolonged and restricted exposure of beetles to PGPR-treated plants.

What mechanism(s) could account for the observed reductions in cucumber beetle density and spread of bacterial wilt in cucumber resulting from PGPR treatment? Published information on the mechanisms of action for PGPR (reviewed by Kloepper et al.1993) suggest that most PGPR strains do not have a single mechanism which completely accounts for the observed beneficial effects on plants, and most underlying mechanisms for biological control by PGPR involve production of bacterial metabolites which have adverse effects on plant pathogens (i.e., siderophores, HCN, antibiotics, lytic enzymes, phytoalexins). Other studies have provided evidence that specific bacteria may systemically elicit physiological changes in plants (Alström 1991, Van Peer et al. 1991, Wei et al. 1991). Systemic disease protection was confirmed in these experiments by application of the inducing agent and the pathogen challenge at different sites on the plant.

A plausible explanation for the reduced numbers of cucumber beetles on PGPR-treated plants in our study is that PGPR induce physiological changes in the plant leading to changes in the production or accumulation of plant allelochemicals acting as beetle attractants, repellents, or feeding stimulants. Diabroticite beetles are attracted to volatiles coming from cucurbit blossoms and probably use these olfactory cues in long-range host finding (Anderson and Metcalf 1986, Lewis et al. 1990). Once on the plant, beetles may leave after

Table 3. Cucumber beetle counts in PGPR-treated field cucumber and in control treatments, 1994

Treatment	Mean no. beetles per plant ± SEM						
	18 May	25 May	1 June	7 June	15 June	22 June	Season avg
Nontreated	0.60 ± 0.14ab	13.30 ± 1.21a	7.73 ± 0.51a	3.80 ± 0.51a	3.60 ± 0.43a	3.47 ± 0.20a	5.42 ± 0.39a
Insecticide control[a]	0.77 ± 0.21a	11.87 ± 1.36a	4.20 ± 0.34b	1.73 ± 0.20b	1.73 ± 0.17b	1.40 ± 0.17b	3.62 ± 0.37b
INR-7	0.23 ± 0.09b	8.43 ± 0.70b	3.93 ± 0.51b	2.17 ± 0.47b	1.63 ± 0.17b	1.37 ± 0.16b	2.96 ± 0.26bc
90-166	0.20 ± 0.07b	6.27 ± 0.60b	3.17 ± 0.52b	1.63 ± 0.23b	1.53 ± 0.17b	1.27 ± 0.19b	2.34 ± 0.20c

Means within columns followed by the same letter are not significantly different (α = 0.05; Newman-Keuls test). Beetle counts (spotted and striped cucumber beetle species combined) made from visual examination of the foliage and flowers on 5 plants per plot, 30 plants per treatment.

[a] Plants sprayed weekly with esfenvalerate insecticide at the rate of 56 g (AI)/ha.

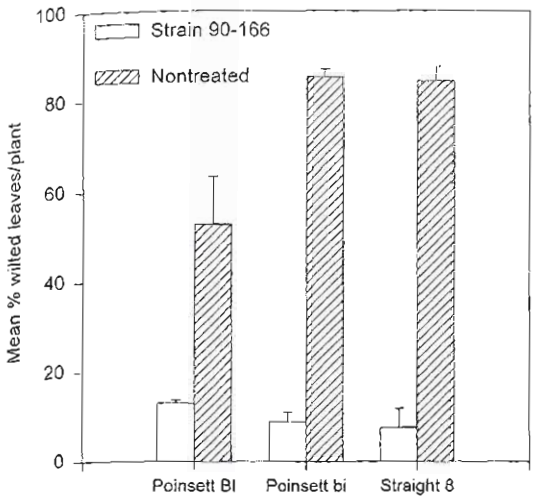

Fig. 1. Comparison of mean percentage of wilted leaves per plant on 'Poinsett' bitter (BI), nonbitter (bi) and 'Straight 8' cucumber plants in no-choice greenhouse cage experiments. Open bars represent plants treated with PGPR strain 90-166 and cross-hatched bars represent nontreated control plants. Spotted cucumber beetles infected with *E. tracheiphila* were released into cages with either PGPR-treated or nontreated plants and allowed to feed for 17–23 d.

1–2 min unless a feeding stimulant is detected (Lewis et al. 1990). Cucurbitacins are a well-known group of bitter tetracyclic triterpenoids that occur mainly in the Cucurbitaceae (Andersen and Metcalf 1986) and produce locomotory arrest and compulsive feeding in the Diabroticite beetles (Chambliss and Jones 1966, Metcalf 1986). Previous studies with *D. u. howardii* and *A. vittatum* support "a strong positive correlation between seedling cucurbitacin content and Diabrotina beetle attacks" (Ferguson et al. 1983). Based on Kogan's (1977) host selection models, Andersen and Metcalf (1986) proposed a model for Diabroticites and cucurbits in which blossom volatiles act in the "host-finding phase" and elicit "orientation to the host plant from a distance," and cucurbitacins act in the "host acceptance stage by stimulating feeding and arresting locomotion." It is conceivable that plant physiological changes associated with PGPR-mediated ISR (i.e., a shift in metabolic pathway to produce other plant defense compounds at the expense of beetle-attractant volatiles or cucurbitacin) resulted in lower numbers of beetles and spread of cucurbit wilt on PGPR-treated plants. To confirm this hypothesis, additional studies must be done to compare known metabolic pathways (i.e., key enzyme, substrate, and end-product concentrations) associated with the production of plant allelochemicals in induced and noninduced plants.

PGPR treatment of crops by seed treatment or transplant drench application is a relatively new biointensive pest management strategy that has great potential for the future. The technology required for formulation of PGPR as commercial seed treatments already exists, as evidenced by the commercial product Kodiak, a *Bacillus subtilus* strain produced by Gustafson (Dallas, TX), and registered as a seed treatment for control of seedling diseases caused by *Rhizoctonia solani* (Backman et al. 1994). Widespread commercial use of PGPR inoculants (mostly *Bacillus* strains) has been reported on many crops grown in China, where PGPR are known as YIB or yield-increasing bacteria (Kloepper 1994).

While most biological control agents have activity against a narrow spectrum of pests or pathogens, a key advantage of PGPR as biological control agents is that PGPR-induced resistance may lead to broad-spectrum protection. This was the case in our experiments where PGPR exhibited dual activity against a plant pathogen and the insect vector. Although it is rare that the efficacy of a biological control agent will equal or exceed control by chemical insecticides, the results of our field experiment demonstrated that PGPR treatment outperformed a recommended, synthetic insecticide for control of cucumber beetle. These results warrant further evaluation of PGPR in other plant pathogen–insect vector systems.

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