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Insect feeding on cucumber mediated by rhizobacteria-induced plant resistance

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Abstract

Select strains of plant growth-promoting rhizobacteria (PGPR) were evaluated in greenhouse experiments with cucumber for induction of resistance against cucumber beetle (*Diabrotica undecimpunctata howardi* Barber) feeding and the beetle-transmitted cucurbit wilt disease. When beetles were given a choice between PGPR-treated and nontreated cucumber, their feeding on stems and cotyledons and the severity of wilt symptoms were significantly lower on PGPR-treated plants. HPLC analysis demonstrated that cotyledons from PGPR-treated plants contained significantly lower concentrations of the cucumber beetle feeding stimulant cucurbitacin than nontreated plants. These results suggest that a mechanism for PGPR-induced resistance against cucumber beetle feeding may involve a change in the metabolic pathway for cucurbitacin synthesis.

Introduction

Contamination of soil and ground water with pesticides poses a growing environmental threat in many agricultural areas. Therefore, a worldwide focus of agricultural research is the development of alternatives to synthetic pesticides for disease and insect management. Research over the past two decades indicates that plants have latent defense mechanisms against pathogens which can be systemically activated upon exposure of plants to stress or infection by pathogens (Kuć, 1985). This phenomenon, called systemic acquired resistance (Ross, 1961) or induced systemic resistance (Tuzun & Kuć, 1985) operates through the activation of multiple defense genes leading to accumulation of defense compounds at sites distant from the point of pathogen attack (Kuć, 1985; Dean & Kuć, 1985). We previously demonstrated that select strains of plant growth-promoting rhizobacteria, when applied to plant seeds or roots, induce systemic resistance to multiple diseases of cucumber, *Cucumus sativus* (Liu et al., 1995a). Here we report for the first time that induced resistance mitigates insect feeding and subsequent transmission of a bacterial plant pathogen in cucumber. This change in feeding behavior was associated with reduced concentration of cucurbitacin, a secondary plant metabolite and insect feeding stimulant.

We first suspected that insect feeding behavior may be altered on cucumber plants treated with specific strains of plant growth-promoting rhizobacteria (PGPR) following field experiments where we unexpectedly observed protection against a naturally-occurring insect-transmitted disease, bacterial wilt of cucurbits (Tuzun & Kloepper, 1995). This systemic disease is 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 (subtribe Diabroticina). *E. tracheiphila* is thought to be entirely dependent on cucumber beetles for inoculation and dissemin-

ation in the field (Agrios, 1978), and a positive, linear relationship exists between cucumber beetle density on plants and disease severity (Yao et al., 1996).

Cucumber beetle feeding behavior is strongly influenced by cucurbitacins, a group of secondary plant metabolites (oxygenated tetracyclic triterpenoids) that occur mainly in the plant family Cucurbitaceae (Chambliss & Jones, 1966). Cucurbitacins are among the most bitter compounds known (Metcalf et al., 1980) and are extremely toxic to most invertebrate and vertebrate herbivores (Nielson et al., 1977; David & Vallance, 1955). An exception are the diabroticine cucumber beetles which can consume cucurbitacins without evidence of acute toxicity. It is theorized that the beetles actually seek out cucurbits for the purpose of acquiring cucurbitacins for protection against predation (Ferguson & Metcalf, 1985; Howe et al., 1976). Cucumber beetles are highly sensitive to cucurbitacins, and even very low concentrations (i.e., 0.001 μg) are effective in producing arrest and compulsive feeding of beetles (Metcalf, 1986). Previous studies with *D. u. howardi* and *A. vittatum* demonstrated 'a strong positive correlation between seedling cucurbitacin content and Diabrotina beetle attacks' (Ferguson et al., 1983). Here we report the results of greenhouse experiments designed 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. Results of experiments to quantify cucurbitacin content in PGPR-treated and nontreated cucumber are also presented.

Materials and methods

Greenhouse experiments. Cage experiments were done in a greenhouse with minimum (night) and maximum (day) temperatures of 24 °C and 28 °C, respectively, and natural (summer) daylight. Cucumber seeds (treated with the PGPR *Bacillus pumilus* strain INR-7 or nontreated) were placed into plastic pots and plants were introduced in cages at the second to fourth true leaf stage (cotyledon leaves were still present). PGPR strains were cultured and maintained as previously described (Liu et al., 1995b), and applied to plants as a seed treatment and root drench (100 ml containing approximately 10^8 cfu/ml) before transplanting. Experiments were done in screen cages designed in a 'cross' arrangement with 4 arms (1.5 m $\times$ 0.5 m $\times$ 0.5 m) with an access door on one side of each arm. At the start of each experiment, 100 spotted cucumber beetles (2 to 4 day-old) (obtained from French Agricultural Research, Lamberton, MN, USA) 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. Plants were artificially infected by injecting 0.5 ml of stem extract from diseased cucumber tissue into the second petiole of cucumber plants. The initial pathogen used to create disease was *E. tracheiphila* culture no. NJH 1302 obtained from M. Havey, Department of Horticulture, University of Wisconsin, Madison. PGPR-treated (seed treatment and transplant drench with INR-7 strain) cucumber 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). Arrangement of treatments among the 4 cage arms was randomized for each experiment. Experiments were repeated twice. PGPR and nontreated means were compared using Student's *t* test analysis.

Cucurbitacin analysis. Fresh or frozen cotyledon leaves from PGPR-treated (with *Bacillus pumilus* strain INR-7 or *Flavomonas oryzihabitans* strain INR-5) or nontreated plants were ground in 100% A.C.S. grade acetonitrile using a 1:2 W:V ratio for the 'Poinsett' plants, and a 1:1 W:V ratio for the 'Straight Eight' plants. 'Poinsett' is a 'bitter' cucumber cultivar with high cucurbitacin content and 'Straight 8' is a commercial cultivar with moderate levels of cucurbitacin. Ground plant material was kept at room temperature for 30 min, then filtered through a 0.45 μm nylon membrane filter. The supernatant (10 μl) was injected directly into the HPLC using a Waters C-18 Novapak 0.8 $\times$ 10 cm column (4 μm diam. particle size). The mobile phase was 60:40 (methanol:H_2O) with a 1 ml/min flow rate. Cucurbitacin 'C', the putative sole cucurbitacin in cucumber, *Cucumus sativus* (Rice et al., 1981), was detected at 230 nm with a Perkin Elmer LC-85B detector. Cucurbitacin 'C' was estimated by peak height measurement and comparison with cucurbitacin 'C' standards obtained from D. Lavie, Dept. Of Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel. Data were analyzed using a single factor ANOVA, and treatment means were compared using the least significant difference test.

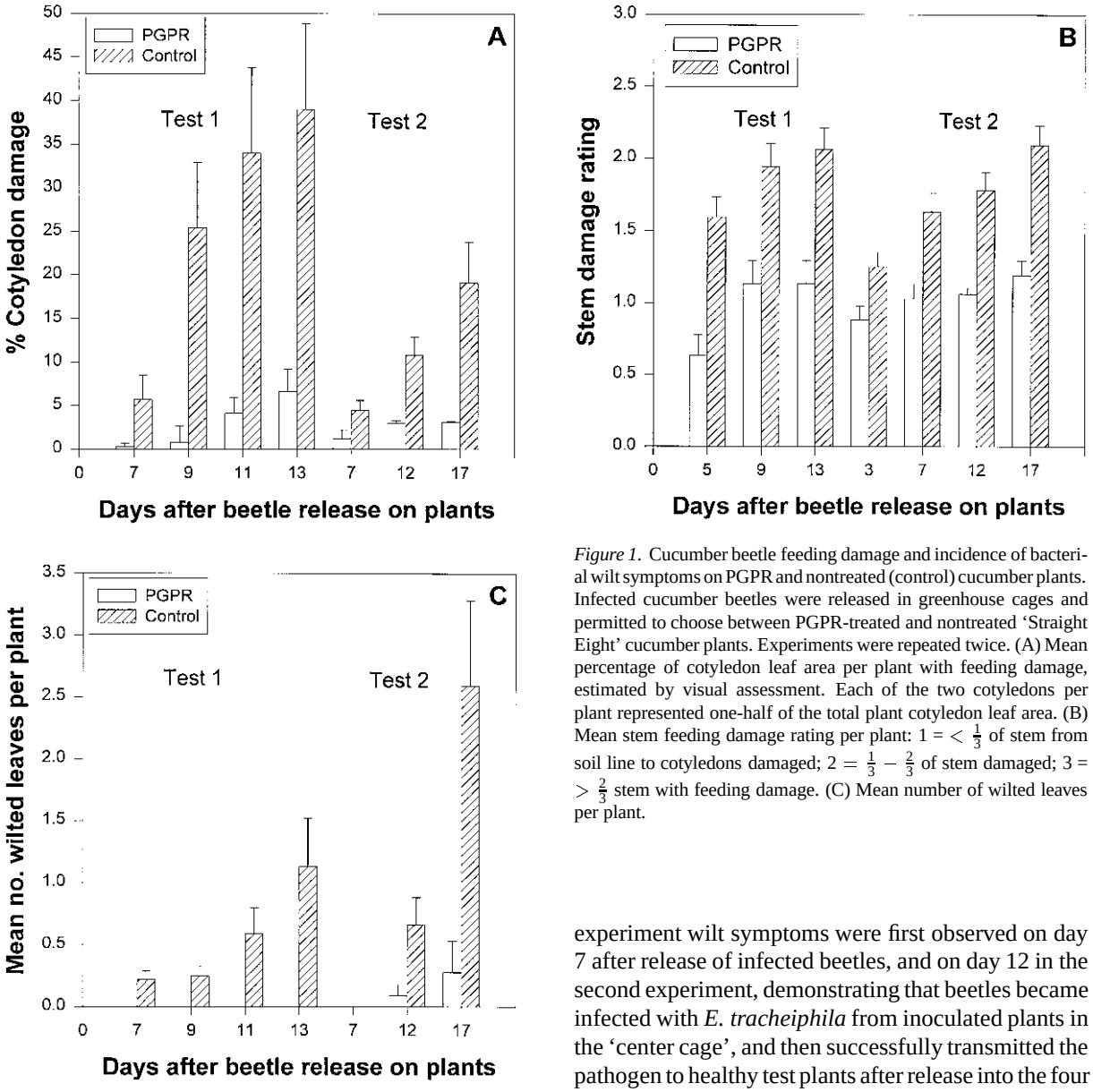

Figure 1. Cucumber beetle feeding damage and incidence of bacterial wilt symptoms on PGPR and nontreated (control) cucumber plants. Infected cucumber beetles were released in greenhouse cages and permitted to choose between PGPR-treated and nontreated 'Straight Eight' cucumber plants. Experiments were repeated twice. (A) Mean percentage of cotyledon leaf area per plant with feeding damage, estimated by visual assessment. Each of the two cotyledons per plant represented one-half of the total plant cotyledon leaf area. (B) Mean stem feeding damage rating per plant: $1 = < \frac{1}{3}$ of stem from soil line to cotyledons damaged; $2 = \frac{1}{3} - \frac{2}{3}$ of stem damaged; $3 = > \frac{2}{3}$ stem with feeding damage. (C) Mean number of wilted leaves per plant.

Results

Greenhouse experiments. Beetle feeding damage on cotyledons was approximately 6- and 10-fold lower on PGPR-treated plants than on nontreated plants at the end of the first and second experiments, respectively (Figure 1A); damage percentages were significantly ($P < 0.05$) different between treatments on all sample dates. Similarly, feeding on stems was significantly less severe on PGPR-treated plants than on nontreated plants in both experiments (Figure 1B). In the first experiment wilt symptoms were first observed on day 7 after release of infected beetles, and on day 12 in the second experiment, demonstrating that beetles became infected with *E. tracheiphila* from inoculated plants in the 'center cage', and then successfully transmitted the pathogen to healthy test plants after release into the four cage 'arms'. An average of 1.13 wilted leaves per plant were recorded on nontreated plants at the end of the first experiment, but wilt symptoms were not observed on PGPR-treated plants (Figure 1C). The average number of wilted leaves per plant at the end of the second experiment was 2.59 on nontreated plants and only 0.28 on PGPR-treated plants. Wilt percentages were significantly different ($P < 0.05$) between treatments on all sample dates. It is not likely that plant infection by *E. tracheiphila* affected beetle feeding preference, because greater feeding damage was observed on the nontreated plants within the first 24 hours after beetle release (before symptom development).

Table 1. Effect of PGPR treatment on cucurbitacin 'C' concentration in 'Poinsett' and 'Straight Eight' cucumber cultivars

PGPR treatment	Mean cucurbitacin concentration (μg/g)	
	Poinsett (bitter) cultivar	Straight Eight cultivar
Bacillus pumilus strain INR-7	117.3 ± 7.1 b	27.1 ± 3.4 c
Flavomonas oryzihabitans strain INR-5	117.9 ± 9.7 b	35.2 ± 4.5 bc
Nontreated	158.6 ± 12.9 a	48.4 ± 3.4 a
LSD, α=0.05	27.3	9.6

Means within columns sharing the same letter are not significantly different ($P>0.05$; least significant difference test). Cucurbitacin 'C' values are μg cucurbitacin/g dry weight plant material. Means derived from 5 replicates per treatment. 'Poinsett' analysis performed on 2 cotyledons from 1 plant/replicate; 'Straight Eight' analysis performed on 4 cotyledons from 2 plants/replicate.

Cucurbitacin analysis. Treatment with PGPR, including the *Bacillus pumilis* strain INR-7 used in the greenhouse cage experiments, significantly decreased the concentration of cucurbitacin 'C' in cotyledons of 'Poinsett' and 'Straight Eight' cucumber (Table 1). The 'Straight Eight' cultivar exhibited the greatest reduction; an average of 44% lower cucurbitacin in INR-7-treated plants compared with nontreated plants.

Discussion

Given that no other plant compounds are known to influence cucumber beetle feeding behavior as strongly as cucurbitacins, the dramatic differences in beetle feeding damage between PGPR-treated and nontreated plants in our greenhouse experiments led us to hypothesize that an unanticipated plant physiological change associated with PGPR-mediated induced resistance was reduced cucurbitacin. The HPLC analysis demonstrated that cucurbitacin levels were reduced as much as 44% on PGPR-treated plants compared with nontreated plants. It is conceivable that beetle feeding behavior was influenced by the induced decrease in cucurbitacin because a strong positive correlation has been established between total cucurbitacin concentration in cucurbit plant tissue and the extent of feeding and aggregation by cucumber beetles (Metcalf et al., 1982; Ferguson et al., 1983). Although the mechanism for PGPR-induced effects on cucurbitacin is not known, a shift in the plant metabolic pathway to produce other plant defense compounds may be involved. Such a change in metabolic pathway was observed in potato where fatty acid elicitors from *Phytophthora infestans* elicited the accumulation of sesquiterpenoid defense compounds (phytoalexins) (Tjamos & Kuć, 1982). This increase was associated with an induced shift in the terpenoid pathway leading to reduced production of steroid glycoalkaloids that are used by the fungus. Previous studies have supported a mechanism for cucurbitacin 'C' biosynthesis in which squalene epoxide is converted by the enzyme squalene synthetase to cucurbitadienol, the simplest tetracyclic triterpene with a cucurbitane skeleton (Balliano et al., 1982). Squalene is also a known precursor of sesquiterpene phytoalexins (Tjamos & Kuć, 1982). Therefore, a similar mechanism for PGPR-induced resistance against cucumber beetle feeding in cucumber may involve a facultative alteration in the metabolic pathway for cucurbitacin synthesis. However, additional studies are needed to compare known metabolic pathways for cucurbitacin synthesis (i.e., key enzyme, substrate and end-procuct concentrations) in induced and non-induced plants before the role of cucurbitacin in PGPR-induced resistance can be confirmed.

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