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Influence of Methyl Bromide Fumigation on Microbe-induced Resistance in Cucumber

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Field experiments were conducted to evaluate growth promotion and induced systemic disease resistance (ISR) in cucumber mediated by plant growth-promoting rhizobacteria (PGPR) with and without methyl bromide soil fumigation. In both fumigated and nonfumigated plots, numbers of cucumber beetles, Acalymma vittata *(F.), and the incidence of bacterial wilt disease, caused by the beetle-transmitted pathogen* Erwinia tracheiphila, *were significantly lower with PGPR treatment compared with the nonbacterized control. However, in PGPR-treated plots, the incidence of bacterial wilt was more than 2-fold lower in the nonfumigated treatments compared with fumigated treatments, indicating that the level of PGPR-mediated ISR was greater without methyl bromide fumigation than with methyl bromide. Cucumber plant growth at 21 days after planting was greater in fumigated plots than in nonfumigated plots; however, plant height values in the nonfumigated, PGPR treatments and the fumigated, PGPR treatments were equivalent. This suggests that PGPR treatment compensated for delayed plant growth that often occurs in nonfumigated soil. These results indicate that, in cucumber production systems, withdrawal of methyl bromide will not negatively impact PGPR-mediated ISR, and also that PGPR may have potential as an alternative to methyl bromide fumigation.*

Keywords: *induced resistance, methyl bromide, plant growth-promoting rhizobacteria (PGPR), cucumber*

INTRODUCTION

Methyl bromide fumigation applied before planting is a widely used practice for disease, nematode and weed control in vegetable production in the USA. The Montreal Protocol, an international treaty developed to protect the earth from the detrimental effects of ozone

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depletion, has called for a 100% reduction in methyl bromide production and use in developed countries by 2005. In 1998, the US Clean Air Act was amended by the US Congress to implement the phasing out of methyl bromide in the US according to the Montreal Protocol schedule. Consequently, vegetable pest managers are desperately seeking alternatives to methyl bromide fumigation. One potential alternative for plant disease management is the use of biological agents, such as plant growth-promoting rhizobacteria (PGPR).

Research over the past two decades has demonstrated that plants have latent defense mechanisms against pathogens, which can be systemically activated by exposure of plants to stress or infection by pathogens. This phenomenon, called systemic acquired resistance or induced systemic resistance, operates through the activation of defense genes and the accumulation of defense compounds at a site distant from the point of pathogen attack (Kessman *et al.*, 1994; van Loon *et al.*, 1998; Buell, 1999). Kloepper and Schroth (1978) first reported that certain root-colonizing bacteria, or PGPR, could promote plant growth, and subsequent studies with PGPR demonstrated control of soilborne pathogens (reviewed in Weller, 1988). More recently, certain PGPR strains have been shown to protect plants through mechanisms associated with induced systemic resistance against pathogens that cause foliar disease symptoms (Alström, 1991; van Peer *et al.*, 1991; Wei *et al.*, 1991). Subsequent research with field-grown cucumber has demonstrated PGPR-induced systemic resistance (ISR) against foliar diseases caused by fungal and bacterial pathogens (Liu *et al.*, 1995a, b). Zehnder *et al.* (1997a, b) demonstrated that treatment of cucumber with PGPR reduced the incidence of wilt symptoms resulting from infection by the bacterial wilt pathogen *Erwinia tracheiphila* (Smith). In these studies, PGPR treatment was also associated with a reduction in numbers of cucumber beetles that are vectors of the bacterial wilt pathogen. The aforementioned studies were conducted using standard vegetable production practices, which include preplant soil fumigation with methyl bromide. Fumigation of cucumber is done primarily to control soilborne pathogens, including damping off caused by *Pythium* and *Rhizoctonia* spp. (Bewick, 1989). These diseases inhibit early plant growth and may delay harvest. As methyl bromide will be unavailable to US vegetable producers after 2005, it is important to determine if PGPR-mediated induced resistance can also occur without methyl bromide fumigation. The objectives of this study were to evaluate PGPR-induced disease resistance in cucumber with and without methyl bromide fumigation, and to determine if PGPR treatment could be used to promote plant growth during early stages when plants are most susceptible to infection by soilborne pathogens.

MATERIALS AND METHODS

Field experiments were conducted over 2 years at the E. V. Smith Horticulture Substation in Shorter, Alabama, USA. Over the previous 6 years, plot land on the Substation was used for production of tomatoes, cucurbits and cabbage in 3-year rotations with a winter grass cover crop planted in the fall; soil type is sandy loam. In the first year, studies were done to compare PGPR treatment, with and without methyl bromide fumigation, to weekly applications of esfenvalerate insecticide (Asana XL™; Dupont, Wilmington, Delaware, USA). Esfenvalerate is routinely targeted against the cucumber beetle vectors of bacterial wilt disease. Treatment plots consisted of two, 9 m long rows of cv. 'Straight 8' cucumber seeded on 22 April. Treatments were replicated four times in a randomized complete block design and included three levels of bacterial wilt control: PGPR treatment; insecticide treatment; untreated control. In fumigated plots, fumigant (335 kg ha^{-1} of 67% methyl bromide + 33% chloropicrin) was injected into the beds followed immediately by application of black plastic mulch. Mulch was also applied to non-fumigated plots but no fumigant was applied in these plots for at least 3 years preceding the study. Fertilization and weed control were done according to local cucumber production practices; fungicides were not applied.

The PGPR strain used in the first year study was *Serratia marcescens* Bizio strain 90–

166, previously shown to induce resistance against bacterial wilt disease in cucumber (Zehnder *et al.*, 1997a, b). PGPR 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 loop-full of bacteria was then transferred to 100 ml of TSB in 1 l flasks and shaken at 150 rpm (24°C) for 24 h. PGPR suspensions were centrifuged at $6000 \times g$ for 5 min and then resuspended in 5 ml of sterile water. Cucumber seeds were dipped into the bacterial suspension or into distilled water (control) immediately before planting into 10 cm^2 plastic pots containing sterilized Promix soilless mix (Preauer Peat Ltd., Riviére-du-Loup, Québec, Canada). A dilute PGPR suspension (100 ml containing approximately 10^8 colony forming units ml^{-1}) was poured into each pot immediately after seeding. Seedlings were transplanted into the field at the second leaf stage. In insecticide plots, esfenvalerate was applied at weekly intervals at the recommended rate of 56 g (a.i.) ha^{-1} using a knapsack sprayer delivering 374 l ha^{-1} at 7 kg cm^{-2} pressure.

Following the initial colonization of plants by striped cucumber beetles, *Acalymma vittata* (F.), numbers were recorded weekly on five randomly chosen plants/plot (20 plants/treatment) on six sample dates. Samples were taken in the morning before 1000 h to facilitate counting on plants before beetles became highly active. Bacterial wilt incidence was determined by recording the number of wilted vines/plant on 16 plants/plot (64 plants/treatment) on a single sample date 10 days before final harvest. Cucumbers were harvested at least twice weekly (total of 10 harvest dates) and weighed to determine fruit yield (cumulative fresh weight) in each plot. Data were analyzed using analysis of variance (ANOVA), and custom hypothesis testing was done to compare treatment means (CONTRAST procedure; SAS Institute, 1990).

The second year experiment was conducted to evaluate PGPR treatment, with and without fumigation, for promotion of early plant growth and for protection against bacterial wilt disease. A different PGPR strain, *Bacillus pumilus* Meyer and Gottheil strain T4, previously shown to induce resistance in cucumber in the greenhouse (unpublished data), was chosen for study in the second year to provide an evaluation under field conditions. Experiments were not designed to compare efficacy of the *S. marcescens* and *B. pumilus* strains. The T4 strain was applied as a soil drench (described above) at planting and at weekly intervals up to 4 weeks after planting. Plots consisted of one, 9 m long row of cv. 'Straight 8' cucumber planted on 27 April. Treatments were replicated six times in a randomized complete block design and included five levels of PGPR treatment (PGPR soil drench at planting plus additional PGPR soil applications at 2, 3 and 4 weeks after planting and a nontreated control), with and without fumigation as described above. Plant height was measured 21 days after planting and the number of wilted vines on 16 plants/plot (64 plants/treatment) recorded at 50, 57, 64, 71 and 78 days after planting. Disease incidence values were converted to area under disease progress curve (AUDPC) values using the following formula:

$$\text{AUDPC} = \Sigma[(0.5)\ (Y_{i+1} + Y_i)\ (T_{i+1} - T_i)]$$

where Y = disease incidence at time T, and i = the time of the assessment (in days numbered sequentially beginning with the initial assessment). Second year data were analyzed using two-factor ANOVA (GLM Procedure; SAS Institute, 1990).

RESULTS AND DISCUSSION

In first year experiment, the mean numbers of cucumber beetles averaged over the season were significantly lower in the PGPR and insecticide treatments than in the control (Figure 1). These results supported earlier findings (Zehnder *et al.*, 1997a) that PGPR treatment was as effective as weekly insecticide applications for control of cucumber beetles. We have previously shown that reduced beetle feeding on PGPR-treated cucumber plants was

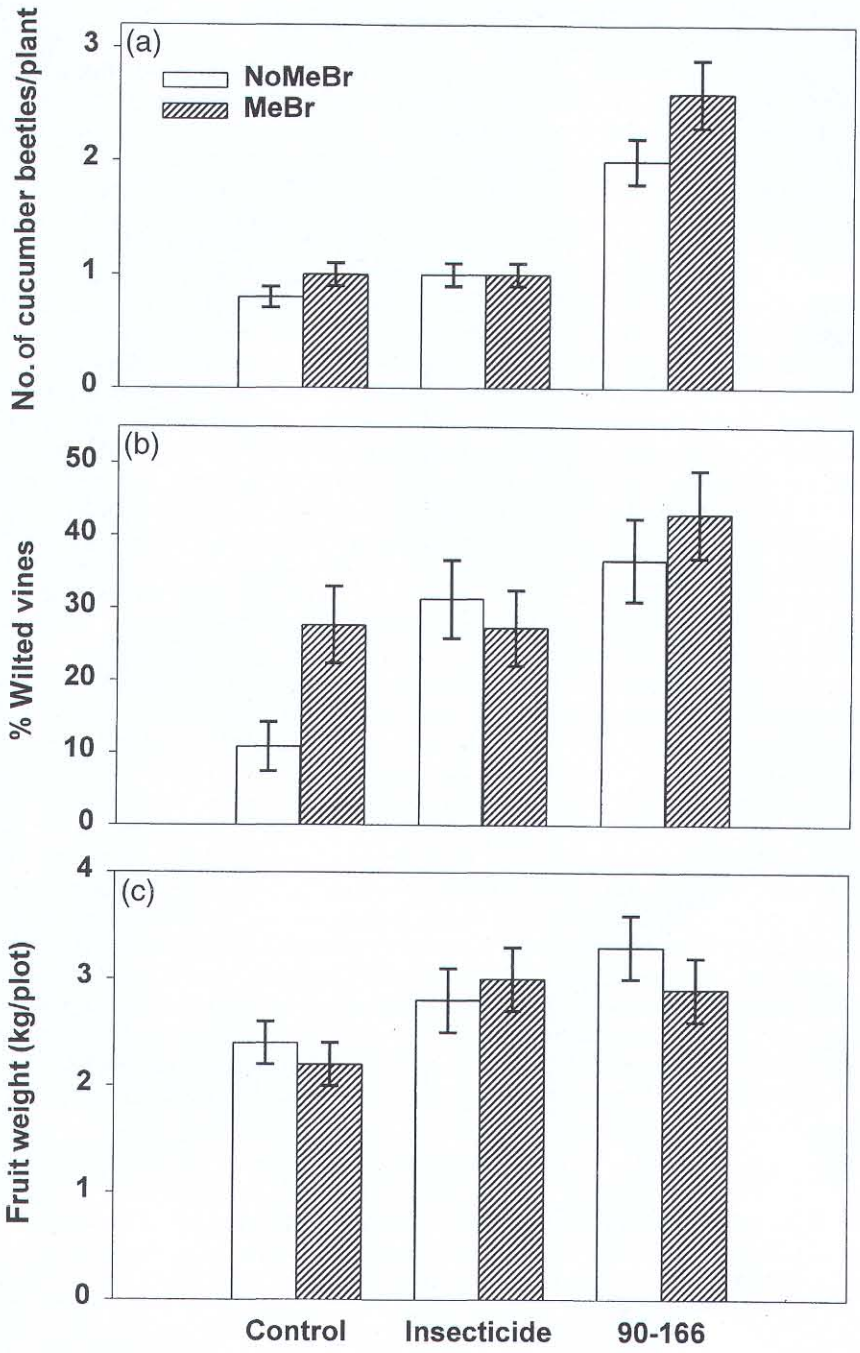

FIGURE 1. First year experiment; number of cucumber beetles/plant (a), percentage of wilted vines/plant (b) and fruit weight/treatment plot (c) in fumigated and nonfumigated control (no PGPR, no insecticide), insecticide (no PGPR) and PGPR (90-166 strain; no insecticide) treatments. Lines within bars represent SE. Beetle numbers represent average numbers/plant over six sample dates. Wilt values represent the average percentage of wilted vines observed and recorded from 64 plants/treatment 10 days before final fruit harvest. Fruit values are the weights (kg) of all marketable fruit/plot averaged over 10 sample dates.

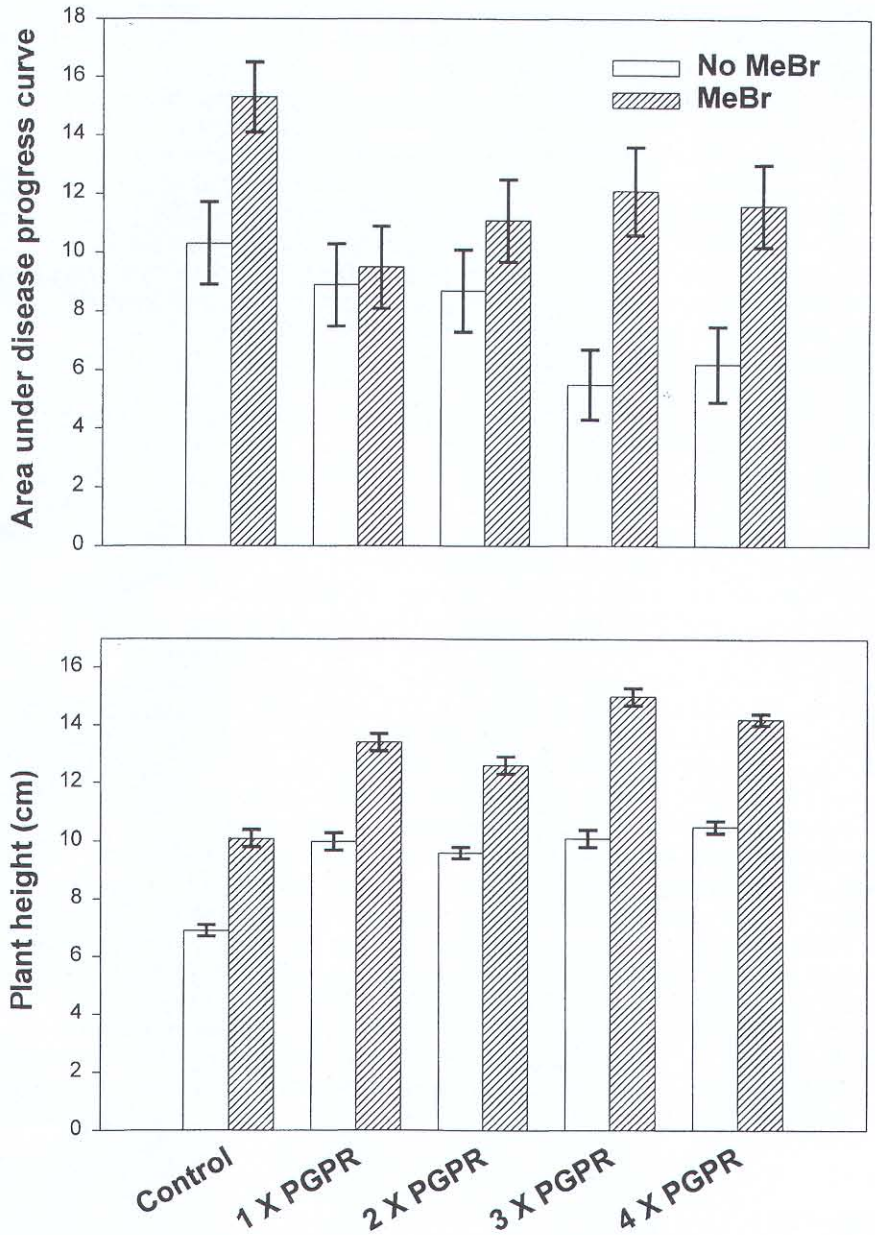

FIGURE 2. Second year experiment; area under disease progress curve (AUDPC) (top) and plant height (bottom) in fumigated and nonfumigated control (no PGPR) and PGPR-treated plots. Lines within bars represent SE. The numbers preceding the PGPR labels indicate the number of PGPR applications. AUDPC values were calculated based on the percentage of wilted vines/plant recorded on 16 plants/plot (64 plants/treatment) over five sample dates. Plant height data are the average height/plant based on measurements taken from 16 plants/plot 21 days after planting.

associated with a decrease in the levels of cucurbitacin 'C' (a powerful cucumber beetle feeding stimulant) on induced plants (Zehnder *et al.*, 1997b). In the PGPR treatment, numbers of beetles were slightly lower in the nonfumigated plots than in the fumigated plots ($P = 0.07$; Figure 1). In both fumigated and nonfumigated plots, disease incidence was significantly lower ($P = 0.0002$) in the PGPR treatment compared with the control (Figure 1). Interestingly, in the PGPR treated plots, the incidence of bacterial wilt was significantly lower in the nonfumigated treatments compared with the fumigated treatments ($P = 0.02$). The percentage of wilted vines was more than 2-fold greater in PGPR-treated fumigated plots compared with PGPR plots without fumigation, indicating that the level of PGPR-mediated ISR was greater without methyl bromide fumigation than with fumigation. In both fumigated and nonfumigated plots, average cucumber yields were significantly greater in the PGPR and insecticide plots than in nontreated plots (Figure 1). In the PGPR-treated plots, yields were higher in nonfumigated treatments than in fumigated treatments, but differences were not significant.

In the second year experiment, the incidence of wilt disease was significantly lower in PGPR-treated cucumber than in cucumber without PGPR treatment (Figure 2). Furthermore, wilt disease was significantly greater in fumigated plots compared with nonfumigated plots ($P = 0.0001$). As in the first year experiment, the incidence of disease was lower in the PGPR, nonfumigated plots than in the PGPR, fumigated plots (PGPR $\times$ fumigation interaction significant at $P = 0.02$).

The second year results also indicated that the at-planting and 'booster' applications of PGPR resulted in increased plant growth in both fumigated and nonfumigated plots, compared with plots where PGPR was not applied (PGPR effects significant at $P = 0.0001$). As expected, plant height values were greatest in fumigated plots (fumigation effects significant at $P = 0.0001$). However, plant height values in the nonfumigated PGPR treatments were equivalent to plant height measurements in the fumigated treatments without PGPR. This suggests that PGPR treatment stimulated plant growth, possibly via growth promotion of the plant, direct negative effects on the soilborne pathogens, indirect effects on the pathogen by induction of disease resistance, or a combination.

These results demonstrate that PGPR induced resistance against bacterial wilt disease occurred in both fumigated and nonfumigated soils, and that the level of disease protection was greater without methyl bromide fumigation. This suggests that soil fumigation has a negative effect on PGPR-induced resistance, possibly by elimination of symbiotic soil microfauna. Further, PGPR treatment compensated for poor early plant growth in nonfumigated soil; evidence that PGPR may be an effective alternative to methyl bromide fumigation in cucumber production. Additional studies are needed to quantify the effects of soil fumigation on PGPR-plant and -rhizosphere interactions, and to examine the mechanisms and possible interrelationships between PGPR-induced disease resistance and growth promotion.

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