Plant and Soil **262:** 277–288, 2004.

Tobacco growth enhancement and blue mold disease protection by rhizobacteria: Relationship between plant growth promotion and systemic disease protection by PGPR strain 90-166

Shouan Zhang[1,2], Munagala S. Reddy[1] & Joseph W. Kloepper[1,3]
[1]*Department of Entomology and Plant Pathology, Alabama Agricultural Experiment Station, Auburn University, Auburn, AL 36849, U.S.A.* [2]*National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL 61604, U.S.A.* [3]*Corresponding author**

Received 16 July 2003. Accepted in revised form 6 November 2003

Key words: biological control, induced systemic resistance, *Peronospora tabacina*, plant growth-promoting rhizobacteria, systemic acquired resistance

Abstract

The effect of plant growth-promoting rhizobacteria (PGPR) on plant growth and systemic protection against blue mold disease of tobacco (*Nicotiana tabacum* L.), caused by *Peronospora tabacina*, was investigated in the greenhouse. Five PGPR strains with known plant growth promotion and induced resistance activities in other crops were used in these studies. PGPR strains were applied as seed treatments alone at planting and in combination with root drenches after planting. When PGPR were applied as seed treatments, PGPR strains 90-166, SE34 and C-9 at 10^9 CFU mL^{-1} increased all or most parameters of plant growth 7 weeks after planting (WAP), while 89B-61 and T4 did not enhance any or few parameters. Seed treatments with PGPR strains 90-166 and C-9 at 10^9 CFU mL^{-1} at 13 WAP resulted in significant disease reduction in blue mold severity compared to the nontreated control. When PGPR were applied as seed treatments and root drenches, all PGPR strains at 10^9 CFU mL^{-1} enhanced tobacco growth compared to the nontreated control at 7 WAP. The time interval between the last PGPR treatment and challenge with *P. tabacina* affected systemic disease protection elicited by some PGPR strains. When the time interval was 8 weeks, 3 PGPR strains 90-166, SE34 and T4 at 10^9 CFU mL^{-1} reduced disease severity, while treatments with all tested PGPR strains resulted in significantly lower disease compared to the nontreated control when it was reduced to 6 weeks. Regression analysis demonstrated a significant relationship between plant growth promotion and systemic protection against blue mold elicited by PGPR strain 90-166. Tobacco growth promotion (X) was calculated by percentage of increase in total fresh plant weight relative to the nontreated control. Systemic protection (Y) against blue mold disease was represented by percentage of decrease in disease severity over the nontreated control. This relationship was best described by the model $Y = -4.48 + 0.37\ X$ ($r^2 = 0.86$, $P = 0.0001$) when strain 90-166 was applied as seed treatments. In the experiment in which strain 90-166 was applied as seed treatments and root drenches, $Y = 6.60 + 0.14\ X$ ($r^2 = 0.88$, $P < 0.0001$) defined this relationship when the time interval was 8 weeks. When the time interval was reduced to 6 weeks, $Y = 12.30 + 0.28\ X$ ($r^2 = 0.80$, $P = 0.0005$) defined the relationship.

Introduction

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil microorganisms that colonize roots and stimulate plant growth. PGPR have been applied to a wide range of crops as biological control agents against fungal, bacterial, and viral pathogens (van Loon et al., 1998). The biological control that results from PGPR is caused by several mechanisms such as competition, antibiosis, and induced resist-

*FAX No: +1-334-844-5067.
E-mail: jkloeppe@acesag.auburn.edu

ance (Kloepper et al., 1980; Kloepper and Schroth, 1981a; Wei et al., 1991; van Loon et al., 1998). Some PGPR produce siderophores that reduce the availability of iron to deleterious microorganisms, resulting in reduced pathogenicity (Kloepper et al., 1980; Schippers, 1988), and/or produce substances harmful to pathogens, such as HCN and antibiotics that suppress deleterious microorganisms in the soil (Thomashow and Weller, 1988, 1995; Schippers, 1992; Maurhofer et al., 1994).

Since the early 1990s, research on mechanisms of biological control by PGPR revealed that some PGPR systemically induce disease resistance against a variety of pathogens in several crops (van Loon et al., 1998), including bean, carnation, cucumber, radish, tobacco (Zhang et al., 1999, 2002), tomato and *Arabidopsis*. PGPR-mediated induced systemic resistance (ISR) has also been effective in field trials with cucumber and tomato against multiple diseases.

Plant growth promotion is another beneficial effect of PGPR and research on PGPR was initially focused on this effect (Burr et al., 1978; Suslow, 1978; Kloepper et al., 1980; Kloepper and Schroth, 1981a,b). PGPR increase seed emergence, plant weight, and yield. In field trials with selected PGPR, emergence increases of 10–40% were reported on canola and soybean, compared to nontreated controls (Kloepper et al., 1986). PGPR increased mid-season plant weight of potato up to 500% (Kloepper et al., 1980; Kloepper and Schroth, 1981b), while yield increases were generally in the 10–15% range. Data from field tests showed that application of PGPR significantly increased yield up to 11% on winter wheat (De Freitas and Germida, 1992) and 18% on canola (De Freitas et al., 1997). Some pseudomonad PGPR increased the fresh weight of cucumber fruit up to 18% (McCullagh et al., 1996).

Mechanisms for PGPR-mediated plant growth promotion include bacterial synthesis of the plant hormones such as cytokinin (Salamone et al., 1997), indole-3-acetic acid (IAA) (Loper and Schroth, 1986; Tang, 1994), and gibberellin (Tang, 1994); breakdown of plant produced ethylene by 1-aminocyclopropane-1-carboxylate deaminase produced in bacteria (Glick, 1995); and increased uptake availability of mineral and N in the soil (Morgenstern and Okon, 1987; Okon et al., 1988). Recently, bacterial produced volatiles such as 3-hydroxy-2-butanone (acetoin) and 2,3-butanediol have been found to trigger plant growth enhancement in *Arabidopsis* (Ryu et al., 2003).

Although there are numerous studies on plant growth promotion and induced resistance elicited by PGPR, little is known about the potential relationship between PGPR-mediated plant growth promotion and induced resistance. Murphy et al. (2003) proved that growth enhancement of tomato by PGPR resulted in disease protection against Cucumber mosaic virus. This research was designed to determine the effect of PGPR on tobacco growth and blue mold disease caused by *Peronospora tabacina*, and finally, for the superior PGPR strain 90-166, we established the possible relationship between growth promotion and systemic disease protection. In our studies, PGPR applied as seed treatments and soil drenches significantly reduced blue mold disease in tobacco under greenhouse conditions (Zhang et al., 1999). Furthermore, enhancement of tobacco growth due to PGPR treatment was evident in the same system.

Materials and methods

PGPR strains and inoculum preparation

Five bacterial strains *Serratia marcescens* 90-166 (Kloepper et al., 1996), *Pseudomonas fluorescens* 89B-61 (Zehnder et al., 1997), *Bacillus pumilus* SE34 (Yao et al., 1997), *Bacillus pumilus* T4 (Zhang et al., 2002) and *Bacillus pasteurii* C-9 (Ji et al., 1997) with reported PGPR activity were used in this study. For long-term storage, bacterial strains were maintained at −80 °C in tryptic soy broth (TSB, Difco Laboratories, Detroit, MI) supplemented with 20% glycerol.

PGPR strains from ultra-cold storage were streaked onto tryptic soy agar (TSA) plates and incubated at 28 °C for 24 h to check for purity. The bacterial inoculum for treatments was prepared by streaking a single colony onto a TSA plate, incubating at 28 °C for 24 h, and removing the resulting bacterial cells from the plate into sterile distilled water. The bacterial suspensions were adjusted to 10^9 CFU mL^{-1} with sterile distilled water and then diluted to appropriate concentrations for experimental use.

Preparation of P. tabacina inoculum

Isolate KY79 of *Peronospora tabacina* (Reuveni et al., 1988) was provided by W.C. Nesmith, University of Kentucky. Inoculum was obtained from freshly sporulating infected leaves of tobacco (*Nicotiana tabacum* L.) cv. Ky14 on 6- to 8-week-old plants at

7 days after inoculation. Sporangia were gently removed into a small quantity of distilled water by using a small paint brush, washed three times with distilled water through a sterile 0.45 μm nylon filter and then resuspended in sterile distilled water to make the inoculum suspensions. The suspensions were adjusted to 10^5 sporangia mL^{-1} by using sterile distilled water under microscopy with a hemocytometer for all experiments.

PGPR applied as seed treatments and root drenches

Tobacco seeds (cv. TN90) (Newton Seed Inc., Hopkinsville, KY) were planted in Speedling potting medium (Speedling Inc., Sun City, FL) in plastic pots (10 × 10 cm). PGPR were applied as seed treatments by pipetting 1 mL of bacterial suspensions on an individual seed at the time of planting. PGPR strains were tested at two different concentrations (10^7 and 10^9 CFU mL^{-1}). One mL of water was applied in the same way as a nontreated control. The assay was designed as a randomized complete block with 11 treatments including a nontreated control with 4 replications per treatment, and 12 seeds per replication. This experiment was repeated two times.

A bioassay was conducted with tobacco cv. TN90 in the greenhouse to test these 5 PGPR strains applied as seed treatments and root drenches for elicitation of growth promotion and systemic disease reduction in blue mold. Seed treatments with PGPR were described as above. Root drenches were applied with 1 mL of bacterial suspensions with the same cell density as seed treatments into the medium at the base of a plant at 2 and 3 WAP, respectively. Each PGPR strain was tested at 10^7 and 10^9 CFU mL^{-1}. Water was applied in the same way as a nontreated control. The assay was designed as a randomized complete block with 11 treatments including a nontreated control, 4 replications per treatment, and 12 seeds per replication. The experiment was conducted twice.

Test for tobacco plant growth promotion and systemic disease protection against blue mold elicited by PGPR

Tobacco plants treated with PGPR as described above were grouped into two sets. One set was used for investigating the effect of PGPR on plant growth. Plant samples were collected at 7 WAP for measuring the area of the third leaf from the bottom, shoot dry weight, root dry weight, and total fresh weight. Subsequently the root/shoot ratio (R/S) was calculated. In the experiment in which PGPR were applied as a seed treatment, emergence was evaluated by counting the final stand of plants at 10 days after planting (DAP). Plant height was also investigated at 3, 4 and 6 WAP.

The second set was used for testing induced systemic disease protection by PGPR against blue mold of tobacco. Tobacco plants were challenged 11 WAP by foliar spray with *P. tabacina* (10^5 sporangia mL^{-1}) until run-off. Inoculated plants were placed in a plastic tent at 22 °C. One to 2 weeks after pathogen challenge, blue mold disease was rated by visually assessing the percentage of leaf surface area with lesions on 3 middle leaves from each tobacco plant.

Another trial was conducted with the same treatments as described above except that the root drenches were conducted at 3 and 4 WAP, and plants were challenged 10 WAP. All these tests were conducted two times in the greenhouse.

Data from repeated greenhouse experiments were pooled, and submitted to analysis of variance using JMP software (SAS Institute Inc., Cary, NC 27513). The significance of plant growth promotion and induced disease protection was determined by the magnitude of the F value ($P = 0.05$). Treatment means were separated by Fisher's protected least significant difference (LSD).

Relationship between growth promotion of tobacco and systemic protection against blue mold elicited by PGPR strain 90-166

PGPR strain 90-166 was tested for potential relationship between growth promotion of tobacco and disease reduction of blue mold disease with tobacco cv. TN90 in the greenhouse. When PGPR strain 90-166 was only applied as seed treatments, 1 mL of the bacterial suspension was pipetted on a seed in Speedling potting medium in plastic pots (10 × 10 cm) at the time of planting. PGPR strain 90-166 was tested at the following concentrations: 1×10^7, 3×10^7, 6×10^7, 1×10^8, 3×10^8, 6×10^8, 1×10^9, 3×10^9, 6×10^9 and 1×10^{10} CFU mL^{-1}. Water was applied as a nontreated control. This assay was designed as a randomized complete block with 11 treatments including nontreated control, 5 replications per treatment, and 12 seeds per replication. Tobacco plants were challenged 11 WAP by foliar sprays with *P. tabacina* (10^5 sporangia mL^{-1}) until run-off. Inoculated plants were placed in a plastic tent at 22 °C.

When PGPR strain 90-166 was applied as a seed treatment in combination with two root drenches as

described previously, two experiments were conducted with different time interval between the last PGPR treatment and the pathogen challenge. One experiment was performed with 8-week inoculation interval, i.e. root drenches were conducted at 2 and 3 WAP, and plants were challenged 11 WAP. The other experiment was 6-week inoculation interval, i.e. the root drenches were conducted at 3 and 4 WAP, and plants were challenged 10 WAP. All others were the same as described above. Each trial was conducted twice.

Fresh weight of tobacco plants was measured at 7 WAP in all three trials. Blue mold disease was rated at 1–2 weeks after pathogen challenge by visually assessing the percentage of leaf surface area with lesions on three middle leaves from each tobacco plant. Three plants in each treatment were randomly sampled for investigating plant growth promotion and systemic disease protection. Data from greenhouse assays were pooled, transformed to the percentage of increase in fresh tobacco plant weight and the percentage of decrease in disease severity of blue mold compared to the nontreated control. Regression analysis was performed using JMP software (SAS Institute Inc., Cary, NC) with the percentage of increase in fresh plant weight as the independent variable (X) and the percentage of decrease in disease severity as a dependent variable (Y) caused by treatments with PGPR strain 90-166. The coefficient of determination (r^2) was calculated for regression models.

Results

Tobacco growth promotion by PGPR applied as seed treatments

Emergence of tobacco plants was not affected by treatments with any of the test PGPR at either concentration except for the treatment with 89B-61 at 10^7 CFU mL^{-1} (data not shown). All five PGPR strains at 10^9 CFU mL^{-1} significantly enhanced plant height, compared to the nontreated control, at 3 and 4 WAP (data not shown). This effect was maintained to 6 WAP with strains 90-166 and C-9.

The effect of PGPR as seed treatments on plant growth measured as different parameters at 7 WAP varied depending on individual PGPR strains and the concentrations applied (Table 1). PGPR strains SE34 and C-9 at 109 CFU mL^{-1} enhanced all parameters of plant growth. Strain 90-166 at 10^9 CFU mL^{-1} increased plant growth in several parameters but not in dry weight of roots. Treatments with strain T4 at 10^9 CFU mL^{-1} increased leaf area of the third leaf. However, treatment with strain 89B-61 did not increase any tobacco growth parameters compared to the nontreated control. At 10^7 CFU mL^{-1}, no PGPR applied as seed treatments promoted plant growth. No other PGPR strains at 10^9 CFU mL^{-1} except C-9 increased plant growth in all parameters compared to 10^7 CFU mL^{-1}. The R/S ratio was not affected by any PGPR treatments (data not shown).

Tobacco growth promotion by PGPR applied as seed treatments and root drenches

When PGPR were applied as seed treatments at planting, and as root drenches either at 2 and 3 WAP (Table 2) or at 3 and 4 WAP (Table 3), the effect of PGPR on tobacco plant growth was evident by 7 WAP. However, effect of plant growth promotion by some PGPR strains decreased over the time. PGPR strains 89B-61 and C-9 at 10^7 CFU mL^{-1} increased the plant growth measured by leaf surface area of the third true leaf, total fresh weight, and shoot dry weight when the time interval between the last PGPR treatment and measurement was 3 weeks (Table 3). However, this did not occur when the time interval was 4 weeks (Table 2). All 5 tested PGPR strains at 10^9 CFU mL^{-1} significantly enhanced tobacco growth compared to the nontreated control. Treatments with some PGPR strains such as T4 (Tables 2 and 3) and C-9 (Table 3) at 10^7 CFU mL^{-1} promoted plant growth with most of the parameters. For each PGPR strain, effect of plant growth promotion by PGPR at 10^9 CFU mL^{-1} was higher than at 10^7 CFU mL^{-1}. No PGPR treatments affected the R/S ratio (data not shown).

Effect of PGPR treatment on severity of blue mold in tobacco applied as seed treatments and root drenches

When PGPR were only applied as seed treatments, disease severity of blue mold was significantly reduced by PGPR strains 90-166 and C-9 at 10^9 CFU mL^{-1} at 13 WAP compared to the nontreated water control (Table 1). Blue mold severity was not reduced by treatments with any other PGPR strains. Only strain 90-166 at 10^9 CFU mL^{-1} reduced disease severity compared to 10^7 CFU mL^{-1}.

In assays where PGPR were used as seed treatments and root drenches, when the time interval between the last PGPR treatment and *P. tabacina* challenge was 8 weeks, PGPR strains 90-166, SE34 and T4 at 10^9 CFU mL^{-1} reduced blue mold severity

Table 1. Influence of PGPR inoculum concentration as seed treatments on tobacco plant growth and blue mold severity under greenhouse conditions

Treatment[w]	Seedling growth[x]					Blue mold severity[y]
	Dose (CFU mL^{-1})	LSA (cm^2)	TFWT (g)	SDWT (g)	RDWT (g)	
90-166	10^9	63.52 ab[z]	9.91 ab	0.806 ab	0.150 abc	38.00 d
	10^7	52.39 bcd	7.95 bcde	0.594 d	0.138 bc	58.25 ab
SE34	10^9	59.87 abc	8.92 abc	0.719 abc	0.163 ab	45.50 bcd
	10^7	48.35 cd	7.66 cde	0.613 cd	0.131 bc	56.25 abc
89B–61	10^9	58.07 abcd	8.69 abcde	0.669 bcd	0.150 abc	59.50 ab
	10^7	45.23 d	6.64 e	0.531 cd	0.131 bc	66.50 a
T4	10^9	63.87 ab	8.90 abcd	0.681 bcd	0.138 bc	55.50 abc
	10^7	46.25 cd	6.83 cde	0.513 d	0.125 bc	60.75 ab
C-9	10^9	71.03 a	10.72 a	0.900 a	0.194 a	41.75 cd
	10^7	50.86 bcd	7.69 cde	0.613 cd	0.125 bc	53.25 abc
Nontreated control		44.18 d	6.71 de	0.513 d	0.113 c	58.25 ab
$LSD_{0.05}$		14.57	2.20	0.189	0.045	15.91

[w]PGPR were applied as seed treatments at two doses on individual seed at the time of planting. Data were from two repeated experiments.
[x]Mean of four replications per treatment, 12 plants per replication. LSA = leaf surface area of the 3rd true leaf; TFWT = total fresh weight; SDWT = shoot dry weight; and RDWT = root dry weight. All growth parameters were measured at 7 WAP.
[y]Blue mold disease severity was visually rated by assessing percentage of leaf surface area covered with lesions at 13 WAP and two weeks after pathogen challenge.
[Z]Means in columns followed by different letters are significantly different at $P = 0.05$ according to Fisher's protected LSD.

Table 2. Influence of PGPR as seed treatments and as root drenches 2 and 3 WAP at two different doses on tobacco plant growth and blue mold severity under greenhouse conditions

Treatment[w]	Plant growth[x]					Blue mold severity[y]
	Dose (CFU mL^{-1})	LSA (cm^2)	TFWT (g)	SDWT (g)	RDWT (g)	
90-166	10^9	22.31 a[z]	2.61 a	0.215 a	0.065 a	10.85 bc
	10^7	5.99 c	0.60 c	0.059 c	0.015 c	16.70 abc
SE34	10^9	20.22 a	2.21 a	0.206 a	0.065 a	12.20 bc
	10^7	5.33 c	0.65 c	0.054 c	0.012 c	26.00 ab
89B-61	10^9	20.90 a	2.51 a	0.200 a	0.059 a	16.45 abc
	10^7	9.98 bc	0.60 c	0.093 bc	0.022 bc	17.45 abc
T4	10^9	22.92 a	2.65 a	0.198 a	0.058 a	9.10 c
	10^7	13.99 b	1.46 b	0.130 b	0.035 b	16.75 abc
C-9	10^9	21.35 a	2.40 a	0.228 a	0.066 a	16.75 abc
	10^7	6.74 c	0.72 bc	0.054 c	0.013 c	25.75 ab
Nontreated control		5.18 c	0.66 c	0.063 c	0.016 c	32.00 a
$LSD_{0.05}$		6.07	0.75	0.057	0.014	15.96

[w]PGPR were applied as seed treatments at two doses on individual seed at the time of planting and root drenches at 2 and 3 WAP. Data were from two repeated experiments.
[x]Mean of four replications per treatment, 12 plants per replication. LSA = leaf surface area of the 3rd true leaf; TFWT = total fresh weight; SDWT = shoot dry weight; and RDWT = root dry weight. All growth parameters were measured at 7 WAP.
[y]Blue mold disease severity was visually rated by assessing percentage of leaf surface area covered with lesions at 13 WAP and two weeks after pathogen challenge.
[z]Means in columns followed by different letters are significantly different at $P = 0.05$ according to Fisher's protected LSD.

Table 3. Influence of PGPR as seed treatments and as root drenches at 3 and 4 WAP at two different doses on tobacco plant growth and blue mold severity under greenhouse conditions

Treatment[w]	Seedling growth[x]					Blue mold severity[y]
	Dose (CFU mL^{-1})	LSA (cm^2)	TFWT (g)	SDWT (g)	RDWT (g)	
90-166	10^9	40.42 a[z]	4.16 a	0.302 a	0.066 a	16.50 bc
	10^7	17.30 ef	1.54 e	0.098 de	0.034 cd	28.25 ab
SE34	10^9	31.06 bcd	3.00 bc	0.208 b	0.058 ab	15.75 bc
	10^7	17.40 ef	1.82 de	0.118 cde	0.034 cd	24.50 abc
89B-61	10^9	35.00 ab	3.31 b	0.223 b	0.067 a	13.25 c
	10^7	27.09 cd	2.72 bc	0.188 b	0.041 bcd	16.25 bc
T4	10^9	31.08 bcd	2.62 bcd	0.173 bc	0.042 bcd	19.50 bc
	10^7	28.55 bcd	2.89 bc	0.204 b	0.050 abc	22.75 abc
C-9	10^9	32.18 bc	3.12 bc	0.202 b	0.056 ab	14.00 c
	10^7	24.14 de	2.40 cd	0.161 bcd	0.044 bcd	25.50 abc
Nontreated control		14.20 f	1.38 e	0.087 e	0.027 d	33.75 a
$LSD_{0.05}$		7.28	0.85	0.066	0.018	12.94

[w]PGPR were applied as seed treatments at two doses on individual seed at the time of planting and root drenches at 3 and 4 WAP. Data were from two repeated experiments.
[x]Mean of four replications per treatment, 12 plants per replication. LSA = leaf surface area of the 3rd true leaf; TFWT = total fresh weight; SDWT = shoot dry weight; and RDWT = root dry weight. All growth parameters were measured at 7 WAP.
[y]Blue mold disease severity was visually rated by assessing percentage of leaf surface area covered with lesions at 12 WAP and two weeks after pathogen challenge.
[z]Means in columns followed by different letters are significantly different at $P = 0.05$ according to Fisher's protected LSD.

Table 4. Influence of PGPR strain 90-166 at various doses as seed treatments on tobacco plant weight and disease severity of blue mold under greenhouse conditions

Dose (CFU mL^{-1})[w]	Total plant fresh weight (g)[x]	% Increase compared to control (%)	Blue mold disease severity[y]	% Decrease compared to control (%)
1.0×10^7	8.90 f[z]	10.3	53.29 ab	1.9
3.0×10^7	9.81 ef	21.5	52.09 ab	4.1
6.0×10^7	10.62 def	31.6	49.00 abc	9.8
1.0×10^8	11.67 cde	44.6	49.65 abc	8.6
3.0×10^8	12.79 bcd	58.5	48.89 abc	10.0
6.0×10^8	12.87 bcd	59.5	42.48 cd	21.8
1.0×10^9	13.12 bc	62.6	46.33 bcd	14.7
3.0×10^9	13.90 bc	72.3	40.14 de	26.1
6.0×10^9	15.13 ab	87.5	40.90 de	24.7
1.0×10^{10}	16.61	99.6	33.57 e	38.2
Nontreated control	8.07 f		54.32 a	
$LSD_{0.05}$	2.41		7.95	

[w]PGPR strain 90-166 was applied as seed treatments at various doses on individual seed at the time of planting. Data were from two repeated experiments.
[x]Mean of 5 replications per treatment and three randomly sampled plants per replication.
[y]Mean of 5 replications per treatment and three plants per replication. Blue mold disease severity was rated by visually assessing percentage of leaf surface area covered with lesions from three middle leaves per plant at 13 WAP and two weeks after pathogen challenge.
[z]Means in columns followed by different letters are significantly different at $P = 0.05$ according to Fisher's protected LSD.

compared to the nontreated control (Table 2). However, when the time interval was reduced to 6 weeks, treatments with all 5 PGPR strains at 10^9 CFU mL^{-1} resulted in lower disease severity compared to the nontreated control (Table 3). At 10^7 CFU mL^{-1}, only strain 89B-61 significantly reduced disease severity of blue mold.

Relationship between growth promotion of tobacco and induced disease reduction elicited by PGPR strain 90-166

Disease reduction in blue mold was proportional to the increase in growth of tobacco plants elicited by treatments of PGPR strain 90-166 in the greenhouse. When strain 90-166 was only applied as a seed treatment, growth of tobacco plants was increased at 10^8 CFU mL^{-1} or higher concentrations, and severity of blue mold was reduced at 6×10^8 CFU mL^{-1} or higher concentrations, compared to the nontreated control (Table 4). When strain 90-166 applied as a seed treatment at planting plus root drenches at 2 and 3 WAP, growth of tobacco plants was increased at 6×10^7 CFU mL^{-1} or higher concentrations. Disease reduction was observed in treatments at 10^8 CFU mL^{-1} or higher concentrations (Table 5) with 8-week inoculation interval. When the inoculation interval was 6 weeks, both growth promotion of tobacco plants and disease reduction in blue mold were noticed at 3×10^7 CFU mL^{-1} or higher concentrations (Table 6).

When PGPR 90-166 was only applied as a seed treatment at planting, the relationship between growth promotion (X) of tobacco plants, represented by the percentage of increase in total fresh plant weight, and systemic disease protection (Y), represented by the percentage of decrease in disease severity of blue mold, was best described by the model $Y = -4.48 + 0.37\ X$ ($r^2 = 0.86$, $P = 0.0001$) (Figure 1A). When PGPR 90-166 was applied as a seed treatment and two soil drenches, $Y = 6.60 + 0.14\ X$ ($r^2 = 0.88$, $P < 0.0001$) best defined the relationship with the time interval between the last PGPR treatment and pathogen challenge was 8 weeks (Figure 1B); when the time interval was reduced to 6 weeks, $Y = 12.30 + 0.28\ X$ ($r^2 = 0.80$, $P = 0.0005$) defined the relationship (Figure 1C). A comparison of two latter regression models revealed that the slopes of the models were significantly different ($P = 0.05$), as determined by the confidence intervals (CI), indicating that when the time interval between the last PGPR treatment and the challenge with *P. tabacina* was 6 weeks (Figure 1C), the percentage of decrease in disease severity of blue mold caused by treatments of PGPR strain 90-166 was significantly higher than that with a time interval of 8 weeks (Figure 1B).

Discussion

A comparison of 90-166 induced plant growth promotion with the decrease in disease severity in tobacco (Figure 1) showed that the capacity to increase plant growth was related to systemic disease protection capacity against blue mold disease. Every time that blue mold severity was reduced, tobacco growth promotion was observed (Tables 1, 2 and 3). However, some PGPR treatments that increased plant growth did not reduce disease severity of blue mold. In addition, tobacco growth promotion was, in general, observed at lower PGPR concentrations than decrease in blue mold severity. These phenomena suggest that antibiosis could be involved in the observed protection against blue mold disease (Sharma and Nowak, 1998; Barka et al., 2002). The strain specific different levels of protection against blue mold under shorter and longer post-inoculation time intervals could be linked to the quorum sensing effects. A certain critical bacterial population density is needed to produce antibiotics and /or signal molecules involved in the systemic protection. For the tested PGPR, it seems likely that the threshold of bacterial populations required for tobacco growth promotion is lower than that for systemic protection. To further confirm phenomena, bacterial populations of PGPR need to be investigated on tobacco roots.

In our studies with PGPR strain 90-166, the PGPR concentrations required for elicitation of systemic disease protection and tobacco growth promotion were higher when 90-166 was only used as a seed treatment compared to those when it was applied as a combination of seed treatments and soil drenches (Tables 4, 5 and 6), indicating that the soil drenches after planting can strengthen the ISR and growth promotion effects. This indirectly reflects that the bacterial population is crucial for elicitation of disease resistance and growth promotion. And, the greater values of intercepts in regressions shown in Figure 1B and Figure 1C relative to that in Figure 1A suggest that treatments with PGPR 90-166 as a seed treatment and soil drenches have the greater potential to reach systemic disease protection. A comparison of the slopes of two regressions in Figure 1B and Figure 1C in-

Table 5. Influence of PGPR strain 90-166 on tobacco growth and on blue mold disease severity as a seed treatment and as two root drenches (8-week inoculation interval)

Dose (CFU mL^{-1})[w]	Total plant fresh weight (g)[x]	% Increase compared to control (%)	Blue mold disease severity[y]	% Decrease compared to control (%)
1.0×10^7	5.62 f[z]	3.6	46.08 ab	5.5
3.0×10^7	6.46 f	19.1	46.52 ab	4.6
6.0×10^7	9.30 e	71.5	42.52 abc	12.8
1.0×10^8	10.79 d	99.1	34.38 bcd	29.5
3.0×10^8	11.99 d	121.2	35.25 bcd	27.7
6.0×10^8	17.60 c	224.8	28.33 d	41.9
1.0×10^9	19.38 b	257.6	30.38 cd	37.7
3.0×10^9	20.22 ab	273.1	25.06 d	48.6
6.0×10^9	20.89 a	285.4	30.91 cd	36.6
1.0×10^{10}	21.33 a	293.5	23.79 d	51.2
Nontreated control	5.42 f		48.76 a	
$LSD_{0.05}$	1.36		13.27	

[w]PGPR strain 90-166 was applied as seed treatments at the time of planting and as root drenches at 2 and 3 WAP. Data were from two repeated experiments.
[x]Mean of 5 replications per treatment and three randomly sampled plants per replication.
[y]Mean of 5 replications per treatment and three plants per replication. Blue mold disease severity was rated by visually assessing percentage of leaf surface area covered with lesions from three middle leaves per plant at 13 WAP and 2 weeks after pathogen challenge.
[z]Means in columns followed by different letters are significantly different at $P = 0.05$ according to Fisher's protected LSD.

Table 6. Effect of PGPR strain 90-166 on tobacco growth and on blue mold disease severity as a seed treatment and two root drenches (6-week inoculation interval)

Dose (CFU mL^{-1})[w]	Total plant fresh weight (g)[x]	% Increase compared to control (%)	Blue mold disease severity[y]	% Decrease compared to control (%)
1.0×10^7	5.57 fg[z]	11.9	38.90 ab	13.3
3.0×10^7	6.56 f	31.7	33.47 bc	25.4
6.0×10^7	8.63 e	73.2	34.10 bc	24.0
1.0×10^8	9.41 de	89.0	30.15 bcd	32.8
3.0×10^8	9.77 cde	96.1	30.11 bcd	32.9
6.0×10^8	10.45 bcd	109.9	25.80 cde	42.5
1.0×10^9	10.93 bde	119.4	20.95 de	53.3
3.0×10^9	11.29 bc	126.7	19.61 de	56.3
6.0×10^9	11.65 b	133.9	17.90 e	60.1
1.0×10^{10}	14.96 a	200.4	18.58 e	58.6
Nontreated control	4.98 g		44.87 a	
$LSD_{0.05}$	1.54		10.72	

[w]PGPR strain 90-166 was applied as seed treatments at the time of planting and root drenches at 3 and 4 WAP. Data were pooled from two repeated experiments.
[x]Mean of 5 replications per treatment and three randomly sampled plants per replication.
[y]Mean of 5 replications per treatment and three plants per replication. Blue mold disease severity was visually rated by assessing percentage of leaf surface area covered with lesions from three middle leaves per plant at 12 WAP and 2 weeks after pathogen challenge.
[z]Means in columns followed by different letters are significantly different at $P = 0.05$ according to Fisher's protected LSD.

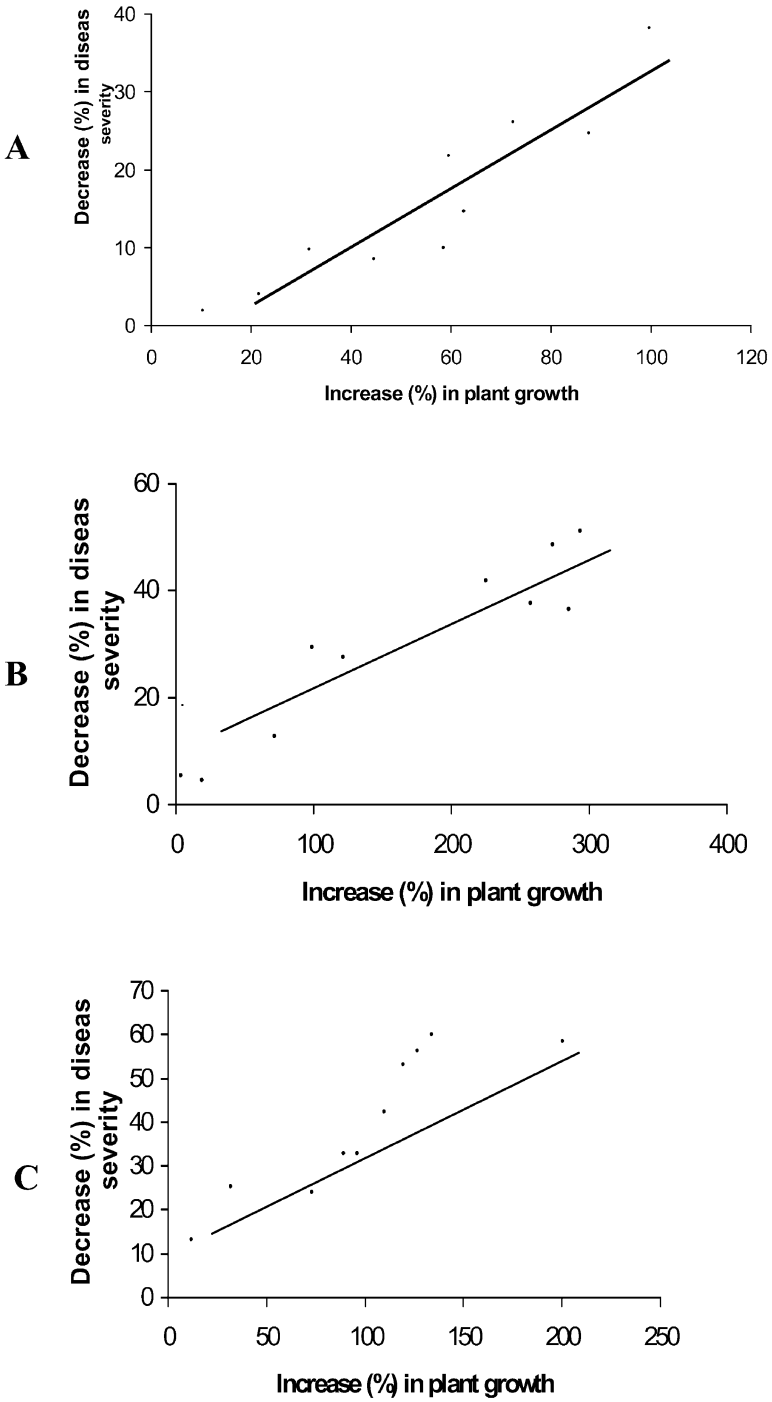

Figure 1. Regression models showing the relationship between tobacco growth promotion and systemic disease protection against blue mold elicited by PGPR strain 90-166. (A) PGPR strain 90-166 was applied as a seed treatment at the time of planting. $Y = -4.48 + 0.37\ X$ ($r^2 = 0.86$, $P = 0.0001$), where X is the percentage of increase in tobacco plant fresh weight compared to the nontreated control, and Y is the percentage of decrease in disease severity of blue mold compared to the nontreated control. (B) PGPR strain 90-166 was applied as a seed treatment at the time of planting and as root drenches at 2 and 3 WAP. The time interval between the last PGPR treatment and pathogen challenge was 8 weeks. $Y = 6.60 + 0.14\ X$ ($r^2 = 0.88$, $P = 0.0001$). (C) PGPR strain 90-166 was applied as a seed treatment at the time of planting and as root drenches at 3 and 4 WAP. The time interval between the last PGPR treatment and pathogen challenge was 6 weeks. $Y = 12.30 + 0.28\ X$ ($r^2 = 0.80$, $P = 0.0005$).

dicated that reduction in blue mold disease by PGPR 90-166 with 6 weeks interval was greater than that with 8 weeks interval period. This may be due to a priming effect on plant defense responses (see below) by PGPR treatments that decreases with increase in time period between the last PGPR inoculation and the pathogen challenge. Priming is a process in which an enhanced capacity in plants achieves to mobilize infection-induced cellular defense responses by treatments including necrotic pathogens, nonpathogenic root-colonizing bacteria and natural or synthetic compounds, such as salicylic acid and β-aminobutyric acid (Conrath et al., 2002). In our previous greenhouse trials (Zhang et al., 1999, 2002), five tested PGPR strains including 90-166, even at 10^7 CFU mL^{-1} (unpublished data), significantly reduced disease severity of blue mold when PGPR were applied as a root drench one week before the pathogen challenge. It is clear that the interval period between last PGPR treatment and the pathogen challenge affects the capacity of systemic blue mold protection. With better understanding of growth promotion and induced systemic protection by PGPR, further research on this possible relationship could be conducted to evaluate plant growth promotion-negative mutants for the capacity to reduce disease, or to investigate induced disease resistance –negative mutants for plant growth promotion effect.

There was no effect of PGPR applied as seed treatments on emergence of tobacco in our studies, while plant growth promotion was observed at 3 WAP. This indicates that physiological and biochemical changes occurring within PGPR-treated tobacco plants after germination led to growth promotion and systemic disease protection. Several studies have demonstrated that PGPR treatments can alter plant physiology, resulting in plant growth stimulation. Tang (1994) reported that some yield-increasing bacteria (YIB) produced plant hormones such as IAA, GA_3 and zeatin that stimulated growth of plants. Peng et al. (1992) used C^{14}-glucose marked YIB to infiltrate corn seeds and found that there were some C^{14}-auxin, gibberellin and zeatin in plants and in bacterial metabolites, indicating that YIB can secrete phytohormones that can be absorbed by plants. Guo et al. (1989) found that activity of zeatin and GA_3 were greatly increased in YIB-treated rice plants 3 weeks after seed treatments.

Simultaneously, inoculation of plants with PGPR can induce defense responses. Induced resistance is associated with an enhanced capacity for the rapid activation of cellular defense responses, which are induced only after contact with a challenging pathogen (Kuć, 1987; Conrath et al., 2002). A phenomenon called priming of defense responses has been described in rhizobacterium-mediated induced systemic resistance (Conrath et al., 2002). The first evidence that priming of plant defense responses is involved in systemically induced resistance was seen from the experiments in which carnation plants develop an increased resistance against *Fusarium oxysporum* f.sp. *dianthi* after root colonization by the PGPR strain *Pseudomonas fluorescens* WCS417. No increase in phytoalexin levels was detected in induced and uninduced plants before the challenge inoculation. However, upon subsequent inoculation with the pathogen, phytoalexin levels in ISR-expressing plants increased significantly faster than those in uninduced plants (van Peer et al., 1991). A beneficial pseudomonad increased a lignin-like component in bean and potato roots (Anderson and Guerra, 1985; Nowak et al., 1990). Rhizobacterium-mediated priming of host cell wall strengthening in pea has also been described by Benhamou et al.(1996). Treatments with a rhizobacterium *Bacillus pumilus* SE34 resulted in ISR against the root rot pathogen *F. oxysporum* f.sp. *pisi*. Colonization of pea root by *Bacillus pumilus* SE34 itself did not induce morphological alterations of root tissue; while, upon challenge with *F. oxysporum*, the root cell walls of induced plants were rapidly strengthened at sites of fungal penetration by appositions of callose and phenolic compounds. Jetiyanon (1997) found that treatment with PGPR strain SE49 in cucumber resulted in rapid enhancement of lignification and increase in total peroxidase activity upon pathogen challenge. In addition, CuZn-Superoxide dismutase (SOD) was substantially increased during early fungal infection in PGPR treatments compared to a nontreated control.

Collectively, the altered plant physiology by PGPR treatments indicates that PGPR may cause a series of physiological and biochemical changes which lead to increased resistance against pathogens or to stimulation of plant growth. Our results indicate that selected PGPR strain 90-166 can stimulate plant growth and result in disease reduction systemically. The correlation between growth promotion and disease reduction is important because visible growth promotion can serve as an indication or prediction of systemic disease protection. Therefore, further research on this correlation with different PGPR is certainly required in the future.

Acknowledgements

We would like to thank K S Park and C M Ryu for many helpful discussions during the research. We acknowledge Kira Bowen and Fenny Dane for their advice on data analysis.

References

Anderson A J and Guerra D 1985 Responses of bean to root colonization with *Pseudomonas putida* in a hydroponic system. Phytopathology 75, 992–995.

Barka E A, Gognies S, Nowak J, Audran J C and Belarbi A 2002 Inhibitory effect of endophyte bacteria on Botrytis cinerea and its influence to promote the grapevine growth. Biol. Control 24, 135–142.

Benhamou N, Kloepper J W, Quadt-Hallmann A and Tuzun S 1996 Induction of defense-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. Plant Physiol. 112, 919–929.

Burr T J, Schroth M N and Suslow T 1978 Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathology 68, 1377–1383.

Conrath U, Pieterse C M J and Mauch-Mani B 2002 Priming in plant-pathogen interactions. Trends Plant Sci. 7, 210–216.

De Freitas J R and Germida J J 1992 Growth promotion of winter wheat by fluorescent *Pseudomonas* under field conditions. Soil Biol. Biochem. 24, 1137–1146.

De Freitas J R, Germida J J and Huatowich G L 1997 Growth and yield response of canola to bacterial inoculants: A three year field assessment. *In* Plant Growth-Promoting Rhizobacteria – Present Status and Future Prospects. Eds. A Ogoshi, K Kobayashi, Y Homma, F Kodama, N Kondo and S Akino. pp. 209–211. Nakanishi Printing, Sapporo, Japan.

Glick B R 1995 The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 41, 109–117.

Guo J C, Le J, Su B L and Wang S L 1989 The influence of hormone within rice seedling treated by YIB. Acta Agric. Universitatis Pekinensis 15, 362.

Jetiyanon K 1997 Interactions between PGPR and cucumber during induced systemic resistance: Recognition and early host defense responses. Ph.D.Thesis, Auburn University, Auburn, AL.

Ji P, Wilson M, Cambell H L and Kloepper J W 1997 Rhizobacterial mediated induced systemic resistance for the control of bacterial speck of fresh-market tomato. *In* Plant Growth-Promoting Rhizobacteria – Present Status and Future Prospects. Eds. A Ogoshi, K Kobayashi, Y Homma, F Kodama, N Kondo and S Akino. pp. 273–276. Nakanishi Printing, Sapporo, Japan.

Kloepper J W, Leong J, Teintze M and Schroth M N 1980 Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286, 885–886.

Kloepper J W, Scher F M, Laliberte M and Tipping B 1986 Emergence-promoting rhizobacteria: Description and implications for agricultures. NATO ASI Series-A Life Sci. 117, 155–164.

Kloepper J W and Schroth M N 1981a Relationship of *in vitro* antibiosis of plant growth-promoting rhizobacteria to plant growth and the displacement of root microflora. Phytopathology 71, 1020–1024.

Kloepper J W and Schroth M N 1981b Development of a powder formulation of rhizobacteria for inoculation of potato seed pieces. Phytopathology 71, 590–592.

Kloepper J W, Zehnder G W, Tuzun S, Murphy J F, Wei G, Yao C and Raupach G 1996 Toward agricultural implementation of PGPR-mediated induced systemic resistance against crop pests. *In* Advances in Biological Control of Plant Diseases. Eds. W Tang, R J Cook and A Rovira. pp. 165–174. China Agricultural University Press, Beijing.

Kuć J 1987 Translated signals for plant immunization. Ann. New York Acad. Sci. 494, 221–223.

Loper J E and Schroth M N 1986 Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. Phytopathology 76, 386–389.

Maurhofer M, Hase C, Meuwly P, Métraux J P and Défago G 1994 Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas flourescens* strain CHAO: Influence of the *gac*A gene and of pyoverdine production. Phytopathology 84, 139–146.

McCullagh M, Utkhed R, Menzies J G, Punja Z K and Paulitz T C 1996 Evaluation of plant growth-promoting rhizobacteria for biological control of Pythium root rot of cucumbers grown in rockwool and effects on yield. Eur. J. Plant Pathol. 102, 747–755.

Morgenstern E and Okon Y 1987 Promotion of plant growth and NO_3^- and Rb^+ uptake in *Sorghum bicolor* × *Sorghum sudanense* inoculated with *Azospirillum brasilense* Cd. Arid Soil Res. Rehabil. 1, 211–217.

Murphy J F, Reddy M S, Ryu C-M, Kloepper J W and Li R 2003 Rhizobacteria-mediated growth promotion of tomato leads to protection against *Cucumber mosaic virus*. Phytopathology 93, 1301–1307.

Nowak J, Frommel M and Lazarovits G 1990 *In vitro* growth promotion of potato by pseudomonad bacterium. J. Cellular Biochem. Suppl.4E, 355.

Okon Y, Fallik E, Sarig S, Yahalom E and Tal S 1988 Plant growth promoting effects of *Azospirillum. In* Nitrogen Fixation: Hundred Years After. Eds. H Botha, F J de Bruijn and W E Newton. pp. 741–746. Gustav Fischer, Stuttgart, West Germany.

Peng G Y, Xiong Y J and Mei R H 1992 Study on the relation between hormones and YIB in plants with isotopic-trace methods. Chinese J. Microecol. 4, 114–116.

Reuveni M, Nesmith W C and Siegel M R 1988 Virulence of an endemic isolate of *Peronospora tabacina* from Texas to *Nicotiana tabacum* and *N. repanda*. Plant Dis. 72, 1024–1027.

Ryu C M, Farag M A, Hu C-H, Reddy M S, Wei H-X, Pare P W and Kloepper J W 2003 Bacterial volatiles promote growth in *Arabidopsis*. Proc. Natl. Acad. Sci. USA 100, 4927–4932.

Salamone I E G, Nelson L and Brown G 1997 Plant growth promotion by *pseudomonas* PGPR cytokinin producers. *In* Plant Growth-Promoting Rhizobacteria – Present Status and Future Prospects. Eds. A Ogoshi, K Kobayashi, Y Homma, F Kodama, N Kondo and S Akino. pp. 316–319. Nakanishi Printing, Sapporo, Japan.

Schippers B 1988 Biological control of pathogens with rhizobacteria. Philos. Trans. R. Soc. Lond. B. 318, 283–293.

Schippers B 1992 Prospects for management of natural suppressiveness to control soilborne pathogens. *In* Biological Control of Plant Diseases, Progress and Challenges for the Future. NATO ASI Series, Series A: Life Sciences, Vol. 230. Eds. E C Tjamos, G C Papavizas and R J Cook. pp. 21–34. Plenum Press, New York.

Sharma V K and Nowak J 1998 Enhancement of verticillium wilt resistance in tomato transplants by in vitro co-culture of seed-

lings with a plant growth promoting rhizobacterium (*Pseudomonas* sp. Strain PsJN). Can. J. Microbiol. 44, 528–536.

Suslow T V 1978 Growth and yield enhancement of sugar beet by pelleting seed with specific *Pseudomonas* spp. (Abstr.). Phytopathol. News 12(9), 40.

Tang W H 1994 Yield-increasing bacteria (YIB) and biocontrol of sheath blight of rice. *In* Improving Plant Productivity with Rhizosphere Bacteria. Eds. M H Ryder, P M Stephens and G D Bowen. pp. 267–273. Commonwealth Scientific and Industrial Research Organization, Adelaide, Australia.

Thomashow L S and Weller D M 1988 Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. J. Bacterial. 170, 3499–3508.

Thomashow L S and Weller D M 1995 Current concepts in the use of introduced bacteria for biological control: Mechanisms and antifungal metabolites. *In* Plant-Microbe Interactions, Vol. 1. Eds. G Stacey and N Keen. pp. 187–235. Chapman and Hall, New York.

van Loon L C, Bakker P A H M and Pieterse C M J 1998 Systemic resistance induced by rhizosphere bacteria, Annu. Rev. Phytopathol. 36, 453–483.

van Peer R, Niemann G J and Schippers B 1991 Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas sp*. strain WCS417r. Phytopathology 8, 728–734.

Wei G, Kloepper J W and Tuzun S 1991 Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. Phytopathology 81, 1508–1512.

Yao C, Zehnder G W, Sikora E, Murphy J and Kloepper J W 1997 Evaluation of induced systemic resistance and plant growth promotion in tomato with selected PGPR strains. *In* Plant Growth-Promoting Rhizobacteria – Present Status and Future Prospects. Eds. A Ogoshi, K Kobayashi, Y Homma, F Kodama, N Kondo and S Akino. pp. 285–288. Nakanishi Printing, Sapporo, Japan.

Zehnder G, Kloepper J W, Yao C and Wei G 1997 Induction of systemic resistance in cucumber against cucumber beetles (Coleoptera: Chrysomelidae) by plant growth-promoting rhizobacteria. J. Econ. Entomol. 90, 391–396.

Zhang S, Reddy M S, Ryu C M and Kloepper J W 1999 Relationship between *in vitro* and *in vivo* testing of PGPR for induced systemic resistance against tobacco blue mold. (Abstr.) Phytopathology 89, S89.

Zhang S, Reddy M S and Kloepper J W 2002 Development of assays for assessing induced systemic resistance by plant growth-promoting rhizobacteria against blue mold of tobacco. Biol. Control 23, 79–86.

Section editor: J.K. Vessey