Photoperiod regulates elicitation of growth promotion but not induced resistance by plant growth-promoting rhizobacteria

J.W. Kloepper, A. Gutiérrez-Estrada, and J.A. McInroy

Abstract: For several years, we have noticed that plant growth-promoting rhizobacteria (PGPR), which consistently promote plant growth in greenhouse tests during spring, summer, and fall, fail to elicit plant growth promotion during the mid-winter under ambient light conditions. This report tests the hypothesis that photoperiod regulates elicitation of growth promotion and induced systemic resistance (ISR) by PGPR. A commercially available formulation of PGPR strains *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a (BioYield®) was used to grow tomato and pepper transplants under short-day (8 h of light) (SD) and long-day (12 h of light) (LD) conditions. Results of many experiments indicated that under LD conditions, BioYield consistently elicited significant increases in root and shoot mass as well as in several parameters of root architecture. However, under SD conditions, such increases were not elicited. Differential root colonization of plants grown under LD and SD conditions and changes in leachate quality partially account for these results. BioYield elicited ISR in tomato and pepper under both LD and SD conditions, indicating that although growth promotion was not elicited under SD conditions, induced resistance was. Overall, the results indicate that PGPR-mediated growth promotion is regulated by photoperiod, while ISR is not.

Key words: rhizobacteria, PGPR, *Bacillus*, induced resistance, growth promotion.

Résumé : Depuis plusieurs années, nous avons remarqué que les rhizobactéries promotrices de la croissance des plantes (plant growth-promoting rhizobacteria-PGPR) qui réussissent régulièrement à promouvoir la croissance des végétaux lors de tests en serre durant le printemps, l'été et l'automne, ne réussissent pas à faire de même au milieu de l'hiver sous des conditions d'illumination ambiante. Cette étude teste l'hypothèse que la photopériode régule le déclenchement la promotion de la croissance et de la résistance systémique induite (RSI) par les PGPR. Une formule disponible commercialement de PGPR constituée des souches *Bacillus subtilis* GB03 et *Bacillus amyloliquefaciens* IN937a (BioYield®) a été utilisée pour faire croître des tomates et des poivrons sous des conditions de luminosité courte (8 h de lumière) ou prolongée (12 h de lumière). Les résultats de plusieurs expériences ont indiqué que sous des conditions de luminosité prolongée, le BioYield induisait de façon régulière des augmentations significatives de la masse des racines et des pousses, ainsi que de plusieurs paramètres relatifs à l'architecture des racines. Cependant, sous des conditions de luminosité courte, une telle augmentation ne se produisait pas. Une colonisation différentielle des racines des plantes cultivées sous des conditions de luminosité courte ou longue, et des changements dans la qualité du lixiviat expliquent en partie ces résultats. Le BioYield a induit une RSI chez la tomate et le poivron tant sous condition de luminosité courte ou prolongée, indiquant que, même si la promotion de la croissance n'était pas induite en luminosité courte, la résistance pouvait l'être. En somme, ces résultats indiquent que la promotion induite par les PGPR est régulée par la photopériode alors que la RSI ne l'est pas.

Mots clés : rhizobactéries, PGPR, *Bacillus*, résistance induite, promotion de la croissance.

[Traduit par la Rédaction]

Introduction

Plant growth-promoting rhizobacteria (PGPR) are root-colonizing bacteria that elicit plant growth promotion or disease control. Implementation of PGPR in agriculture and horticulture has begun with the marketing of products containing well-tested PGPR strains. One PGPR product that has been reported in the literature is BioYield®, which contains spore preparations of PGPR strains *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a on chitosan flakes (Kloepper et al. 2004*a*). When applied to soilless media used to grow transplanted vegetables, BioYield has been reported to increase root and shoot mass, stem caliper, and the root to shoot ratio of tomato, pepper, cucumber, tobacco, and melons (Kokalis-Burelle et al. 2003; Kloepper et al. 2004*a*).

When BioYield-treated seedlings were transplanted in field trials, increased transplant survival and vigor, improved root condition, and decreased root colonization by *Fusarium*

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Table 1. Winter test with supplemental light: day length effect on growth promotion with BioYield®.

	Long-day conditions[a]				Short-day conditions[a]			
	Root		Shoot		Root		Shoot	
Treatment[b]	Fresh mass (g)	Dry mass (g)	Fresh mass (g)	Dry mass (g)	Fresh mass (g)	Dry mass (g)	Fresh mass (g)	Dry mass (g)
Pepper								
BioYield 1:40	0.15	0.008	0.15	0.015	0.09	0.0040	0.19	0.017
BioYield 1:100	0.21	0.011	0.24	0.025	0.05	0.0034	0.13	0.015
Control	0.11	0.006	0.09	0.008	0.09	0.0040	0.15	0.015
$LSD_{0.05}$	0.06	0.003	0.03	0.003	0.02	0.0012	0.03	0.003
Tomato								
BioYield 1:40	0.29	0.017	0.38	0.059	0.08	0.004	0.17	0.017
BioYield 1:100	0.41	0.016	0.36	0.056	0.12	0.003	0.21	0.015
Control	0.14	0.007	0.10	0.022	0.10	0.004	0.18	0.015
$LSD_{0.05}$	0.09	0.003	0.09	0.010	0.07	0.002	0.04	0.003

[a]Long day indicates 12 h light period; short day indicates 8 h light period. Values shown are the means of 10 replicate plants per treatment.

[b]BioYield® contains *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a. It was mixed into soilless potting media at a ratio of 1:40 or 1:100 (*v*/*v*) prior to planting seeds in transplant trays.

and *Pythium* spp. were noted on tomato and pepper (Kokalis-Burelle et al. 2003). In a separate study on tomato (Kloepper et al. 2004*a*), BioYield-treated tomato transplants (treatment with chitosan + GB03 + IN937a) had reduced gall ratings caused by root-knot nematodes (*Meloidogyne incognita*). Hence, with BioYield, there is a positive relationship between plant growth promotion at the seedling stage in the greenhouse and subsequent performance of plants transplanted to the field. Therefore, growth promotion in the greenhouse has been used to optimize BioYield application rates and examine the range of plant varieties amenable to PGPR-mediated growth promotion. However, such greenhouse studies have been frustrated by the following scenario. Throughout the spring, summer, and fall, significant growth promotion was noted in nearly all tests with BioYield on tomato, bell pepper, tobacco, and cucumber (Kloepper et al. 2004*a*). In the winter, however, conducting the same experiments often resulted in lack of significant growth promotion. Because temperature in the greenhouse complex varied by only about 5 °C from summer to winter, we suspected that the lack of growth promotion in the winter was related to short photoperiods.

Photoperiod affects the processes of photosynthesis, translocation, and respiration in plants, thereby affecting the quantity and quality of root leachates (Hale et al. 1971). Several studies reviewed in Gibon et al. (2004) indicate that under short-day (SD) conditions, plants partition more of their photoassimilates into starch in leaves than as carbohydrates that can be exported to roots. In studies with *Arabidopsis*, Gibon et al. (2004) reported that under long-day (LD) (12 h of light) conditions, starch accumulated in leaves during the day and was mobilized to carbohydrates during the night, when the starch concentration of leaves fell. Under SD (8 h of light) conditions, starch also accumulated during the day, but its mobilization to carbohydrates during the night was less than under LD conditions so that at the end of the night, there was still considerable starch in the leaves. The level of sugars in leaves at the end of the night was 50% lower under SD than under LD conditions. The difference in sugar levels in leaves of plants grown under LD and SD conditions was also noted in roots. With LD conditions, the diurnal concentration of sucrose in roots was relatively stable, while with SD conditions, sucrose levels were low at the end of the night, increased rapidly after illumination, and then decreased in the second part of the day. This change in the sucrose levels of roots under LD and SD conditions could likely be reflected in different patterns of root exudation. In a study with hydroponically grown cucumber, Pramanik et al. (2000) reported that root leachates were chemically different between plants grown under SD (10 h of light) and LD (14 h of light) conditions. In the same study, root and shoot mass were significantly greater for plants grown under LD conditions than plants grown under SD conditions.

While much work has been done to characterize plant metabolic responses to photoperiod, there are few reports describing how plant colonization by microorganisms is affected by photoperiod. Tsror (2004) studied the effect of photoperiod on the severity of potato black dot caused by *Colletotrichum coccodes*. Fungal colonization of stem segments was significantly greater on four potato cultivars under SD (8 h of light) than under LD (16 h of light) conditions. The number of sclerotia on roots was also greater under SD than under LD conditions. In contrast with these results indicating greater colonization with short days, Bodelier et al. (1998) found that root colonization with bacteria was greater under LD (20 h of light) than under SD (12 h of light) conditions. In this study, which was part of a comprehensive investigation into interactions between nitrifying and denitrifying bacteria, root colonization of the nitrifying bacteria *Nitrosomonas europaea* and *Nitrobacter winogradskyi* and the denitrifying bacterium *Pseudomonas chlororaphis* was assessed on *Glyceria maxima* in microcosms. Plant biomass and populations of *P. chlororaphis* were significantly greater under LD than under SD conditions. With long days, denitrifying activities by the plant were increased and populations of *N. winogradskyi* were decreased. Hence, increased plant growth with long days favored the population of one root-associated bacterium and decreased the population of another bacterium.

Table 2. Summer test: day length effect on growth promotion with BioYield®.

	Long-day conditions[a]				Short-day conditions[a]			
	Root		Shoot		Root		Shoot	
Treatment[b]	Fresh mass (g)	Dry mass (g)	Fresh mass (g)	Dry mass (g)	Fresh mass (g)	Dry mass (g)	Fresh mass (g)	Dry mass (g)
Pepper								
BioYield 1:40	0.43	0.031	1.17	0.09	0.17	0.012	0.82	0.06
BioYield 1:100	0.47	0.043	1.03	0.08	0.20	0.013	0.89	0.07
Control	0.28	0.021	0.93	0.08	0.22	0.013	0.95	0.07
$LSD_{0.05}$	0.07	0.007	0.15	0.018	0.05	0.004	0.13	0.11
Tomato								
BioYield 1:40	0.20	0.018	0.61	0.052	0.19	0.012	0.49	0.044
BioYield 1:100	0.36	0.020	0.71	0.060	0.22	0.014	0.54	0.042
Control	0.15	0.010	0.48	0.041	0.22	0.013	0.62	0.048
$LSD_{0.05}$	0.04	0.003	0.05	0.004	0.05	0.003	0.08	0.007
Marigold								
BioYield 1:40	0.32	0.018	0.67	0.054	0.06	0.004	0.33	0.026
Control	0.12	0.008	0.42	0.042	0.12	0.008	0.37	0.028
$LSD_{0.05}$	0.05	0.003	0.06	0.006	0.04	0.002	0.03	0.003
Cucumber								
BioYield 1:40	1.10	0.058	1.54	0.15	1.61	0.015	1.61	0.10
Control	0.77	0.028	1.36	0.14	1.88	0.019	1.88	0.11
$LSD_{0.05}$	0.22	0.010	0.15	0.01	0.18	0.004	0.18	0.015

[a]Long day indicates 12 h light period; short day indicates 8 h light period. Values shown are the means of 10 replicate plants per treatment.
[b]BioYield® contains *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a. It was mixed into soilless potting media at a ratio of 1:40 or 1:100 (*v*/*v*) prior to planting seeds in transplant trays.

Based on our past observations and the publications cited above, we formed the following hypotheses: (*i*) elicitation of plant growth promotion and induced systemic resistance (ISR) by a model PGPR system are regulated by photoperiod and (*ii*) differential root colonization by PGPR under LD and SD conditions and changes in root leachates will help explain photoregulation of growth promotion.

Materials and methods

Application of BioYield

BioYield, which contains spore preparations of PGPR strains *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a on chitosan, was obtained from Gustafson, LLC (Dallas, Texas). For the experiments reported here, BioYield was mixed into ProMix soilless potting mix (Premier BioTech, Riviere-du-Loup, Quebec) at the recommended rate for tomato of 1:40 (*v*/*v*) and the recommended rate for pepper of 1:100. Seeds of tomato cultivar Juliet or bell pepper cultivar California Wonder (Park Seed, Anderson, South Carolina) were planted in soilless media with and without BioYield in 128 Speedling transplant trays (Speedling, Inc., Plant City, Florida).

Influence of day length on PGPR-mediated plant growth promotion

Winter tests

Two experiments were conducted, one with pepper and one with tomato, to determine the effects of BioYield on plant mass under LD and SD conditions. SD conditions were 8 h of ambient light in a greenhouse during the winter. LD conditions of 12 h of light were obtained by using supplemental lighting (400 μmol/(s·m^2)). When the supplemental lights were on, room temperature at the height of plants averaged 2 °C warmer than without lights. Each experiment consisted of three treatments: BioYield at a ratio of 1:40, BioYield at a ratio of 1:100, and a nontreated control. Each treatment consisted of 10 replicate plants. Plants were sampled at 3 weeks after planting by removing them from the transplant trays, washing to remove soilless media, blotting dry, and weighing the shoots and roots. Dry masses were also determined for each plant. Masses were analyzed with ANOVA, and when a significant *F* value was determined, treatment means were separated using LSD at $P = 0.05$. Each of the experiments was conducted twice with similar results.

Summer tests

Because supplemental lighting in the winter tests increased air temperature slightly, further tests were done around the summer equinox. In these tests, SD conditions were created by covering plants with a cardboard box daily to allow an 8 h photoperiod. Separate experiments were conducted on pepper, tomato, marigold, and cucumber. For experiments with pepper and tomato, the treatments were the same as in the winter tests. With marigold and cucumber, treatments included BioYield at a ratio of 1:40 and a nontreated control. Each experiment was conducted twice with the same results. Fresh and dry masses were assessed after 3 weeks for pepper, 17 days for tomato, 15 days for marigold, and 14 days for cucumber.

Table 3. Effects of BioYield® on root architecture under long-day and short-day conditions.[a]

Treatment[b]	Root surface area (cm^2)	Projected area (cm^2)	Root volume (cm^3)	Mean root diameter (mm)	No. of root tips	Length of total roots (cm)	Width: Roots with diameter of 0–0.5 mm	Width: Roots with diameter of 0.5–1.0 mm
Long-day conditions								
Pepper								
BioYield 1:40	28.55	9.08	1.56	2.22	147.3	42.2	10.9	9.43
BioYield 1:100	33.46	10.65	1.64	1.97	209.1	54.6	16.2	13.7
Control	26.59	8.53	1.46	2.22	94.2	39.6	10.3	8.9
$LSD_{0.05}$	3.86	1.21	0.18	0.24	53.2	9.4	4.0	3.78
Tomato								
BioYield 1:40	35.06	11.12	1.38	1.59	220.1	73.4	24.1	25.5
BioYield 1:100	35.03	11.34	1.38	1.58	226.1	74.0	25.0	23.6
Control	29.85	9.50	1.52	2.04	125.6	47.3	13.5	11.5
$LSD_{0.05}$	4.22	1.43	0.17	0.24	45.6	17.4	6.5	9.8
Short-day conditions								
Pepper								
BioYield 1:40	23.87	7.72	1.58	2.65	73.3	29.4	5.1	4.2
BioYield 1:100	21.97	6.98	1.45	2.66	93.2	26.4	5.3	4.6
Control	23.22	7.32	1.40	2.43	80.8	30.1	7.5	4.4
$LSD_{0.05}$	1.65	0.61	1.00	0.22	18.5	4.4	2.4	2.4
Tomato								
BioYield 1:40	26.90	8.56	0.99	1.47	102.3	26.9	19.2	23.2
BioYield 1:100	23.96	7.62	1.08	1.83	83.7	23.9	16.7	12.6
Control	28.60	8.06	1.17	1.61	101.0	28.6	13.3	28.6
$LSD_{0.05}$	3.73	1.22	0.15	0.35	20.7	3.7	6.7	3.7

[a]Root architecture was determined using WinRhizo analysis of roots. Long day indicates 12 h light period; short day indicates 8 h light period. Values shown are the means of 10 replicate plants per treatment.

[b]BioYield® contains *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a. It was mixed into soilless potting media at a ratio of 1:40 or 1:100 (*v*/*v*) prior to planting seeds in transplant trays.

Influence of day length on changes in root architecture elicited by BioYield

Following the observation that under LD conditions, total root mass was consistently increased by BioYield, we designed experiments to obtain more precise information on how roots are affected by BioYield under LD and SD conditions. Accordingly, experiments were conducted to determine the influence of day length on changes in root architecture elicited by BioYield. Separate experiments were done on pepper and tomato in both the winter and the summer as described above. The treatments, replications, and growth conditions were the same as in the previous experiments. After washing roots, an analysis of root architecture was made on each plant's root system using the system of Régent Instruments, Inc. (Sainte-Foy, Quebec), which consists of scanner model LA 1600+ and WinRhizo software (version 2004a). Data from the resulting analyses were collected for eight parameters: root surface area, projected area, root volume, mean root diameter, number of root tips, total root length, and length of the two smallest diameter classes of roots (0–0.5 and 0.5–1.0 mm). All data were analyzed using ANOVA and treatment means were compared with the control mean using LSD at $P = 0.05$. Each experiment was conducted two times with similar results. Results from one trial of the winter tests are presented.

Influence of day length on elicitation of ISR by PGPR contained in BioYield

Experiments were designed to test the hypothesis that elicitation of ISR by BioYield was regulated by photoperiod. One experiment was conducted on tomato and one on pepper. Seeds were planted and treated with both rates of BioYield and maintained under LD and SD conditions as described previously; a nontreated control was included for each condition of day length. At 4 weeks after planting, eight seedlings of each treatment were transplanted into 0.5 L (10 cm^2) pots.

The disease evaluated was bacterial spot of tomato and pepper caused by *Xanthomonas axonopodis* pv. *vesicatoria*. The pathogen was obtained from the culture collection of the Department of Entomology and Plant Pathology of Auburn University, Auburn, Alabama. The bacterium was grown on tryptic soy agar (TSA) at 28 °C for 24 h and used to prepare a cell suspension adjusted to OD_{540} = 0.2 (log 8.0 CFU/mL) and suspended in an 0.01 mol/L phosphate buffer at pH 7.0 using distilled water. One week after transplanting, eight replicate plants per treatment

Table 4. Day length effect on induced systemic resistance by BioYield® against bacterial spot disease.[a]

	Mean no. of leaf spots per leaf[b]					
	Long-day conditions[c]			Short-day conditions[c]		
Treatment	Upper leaf	Lower leaf	Both leaves	Upper leaf	Lower leaf	Both leaves
Pepper						
BioYield 1:40	173.8	196.9	185.3	169.1	188.0	175.5
BioYield 1:100	162.9	168.6	165.7	163.6	177.7	170.6
Control	235.9	250.4	243.1	247.5	265.9	256.7
$LSD_{0.05}$	47.1	58.3	57.1	58.0	62.5	65.6
Tomato						
BioYield 1:40	35.1	37.4	36.2	14.1	23.0	18.6
BioYield 1:100	68.9	33.6	51.2	15.6	14.1	14.8
Control	138.2	107.9	123.0	215.0	177.4	196.2
$LSD_{0.05}$	65.0	38.4	39.6	72.0	76.1	59.5

[a]Bacterial spot disease is caused by *Xanthomonas axonopodis* pv. *vesicatoria*.
[b]Values shown are the means from two upper leaves and two lower leaves per plant with eight replicate plants per treatment.
[c]Long day indicates 12 h light period; short day indicates 8 h light period.

were challenged with the pathogen by spraying leaves evenly with the bacterial suspension. A plastic bag was then placed over each plant to maintain high relative humidity conditions required for infection of the pathogen. After 72 h, the plastic bags were removed. At 10 days after pathogen challenge, disease severity was rated by counting the number of bacterial lesions on two bottom leaves and two uppermost fully expanded leaves of each plant. The mean number of lesions on upper leaves and on lower leaves and combined values were calculated for each plant. These numbers were analyzed using ANOVA. Treatment means were separated using the LSD test at $P = 0.05$. With both tomato and pepper, the experiment was conducted twice with similar results, and results from one trial per crop are presented.

Influence of day length on root colonization by PGPR

An experiment was designed to determine if the observed lack of growth promotion by BioYield under SD conditions related to differential colonization of roots by the PGPR strains in the product under LD and SD conditions. Spontaneous mutants of *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a were selected for resistance to 100 μg/mL of rifampicin in broth culture. Mutants were compared with the wild-type strains for similarity of growth rates on TSA. The selected mutants were spread-plated on TSA amended with 100 μg/mL rifampicin (TSA-rif) and incubated for 48 h at 30 °C. The resulting bacterial lawns were scraped off plates using 10 mL of sterile water, an additional 10 mL of sterile water was added, and 50 μL of the suspension was pipetted onto each seed of tomato planted into transplant trays as described previously. Treatments included the two PGPR strains and a water control, each under SD and LD conditions as described previously. At 1, 2, and 3 weeks after planting, six replicate plants per treatment from LD and SD conditions were removed from the transplant tray and shaken to remove loosely adhering soilless media. Roots were cut from the stem, weighed, and placed in sterile water blanks. Serial 10-fold dilutions were prepared and plated onto TSA-rif. After incubation for 48 h, colonies were enumerated and the CFU per gram of root calculated. Data were log transformed prior to analysis by ANOVA and calculation of LSD at $P = 0.05$ to compare means of colonization on plants under LD and SD conditions.

Quality of root leachates from plants under LD and SD conditions

To determine if day length affected the quality of root leachates through supporting bacterial growth, two experiments were conducted: one with leachates from pepper and one with leachates from tomato. Seeds were planted in soilless media without BioYield and were maintained under LD or SD conditions for 3 weeks, and then, 25 mL of water was poured into each cell of the transplant tray (where each cell contained one seedling). The water running through the bottom hole of each cell was collected as the leachates for investigation. The leachates were filter sterilized by passing through a 0.22 μm sterile filter unit, and 15 mL aliquots were placed into 50 mL Erlenmeyer flasks. For each treatment, six replicate flasks were used. Strains GB03 and IN937a were grown in tryptic soy broth for 24 h, and 20 μL of culture was added to each flask of leachates to yield an initial inoculum density of log 3.69 CFU/mL. The inoculated flasks were maintained at room temperature with shaking at 200 r/min.

Growth of each strain in leachates of tomato and pepper was compared between plants grown under LD and SD conditions. Growth was determined by plating 50 μL from each flask onto TSA at various sample times to enumerate CFU per millilitre. Sample times for pepper leachates were 16, 22, 26, 32, 42, and 50 h after inoculation. Data were analyzed by conducting ANOVA analysis at each sample time for a population of one strain in leachates collected from plants under LD and SD conditions.

Results

Influence of day length on PGPR-mediated plant growth promotion

Winter tests

BioYield promoted growth of pepper and tomato (Table 1)

Table 5. Colonization of tomato roots by plant growth-promoting rhizobacteria (PGPR) strains in BioYield® under long-day and short-day conditions.[a]

PGPR strain	Mean log CFU/g root		
	1 week after planting	2 weeks after planting	3 weeks after planting
Bacillus subtilis GB03			
Long day	2.49	3.06	2.98
Short day	1.40	2.92	2.98
$LSD_{0.05}$	1.09	0.31	0.41
Bacillus amyloliquefaciens IN937a			
Long day	3.51	3.79	3.58
Short day	3.01	3.46	3.78
$LSD_{0.05}$	0.37	0.25	0.24

[a]Long day indicates 12 h light period; short day indicates 8 h light period. Values shown are the means of six replicate plants per treatment.

Fig. 1. Growth of strains GB03 (A) and IN937a (B) in leachates of pepper under long-day (LD) and short-day (SD) conditions. Error bars represent the standard deviation.

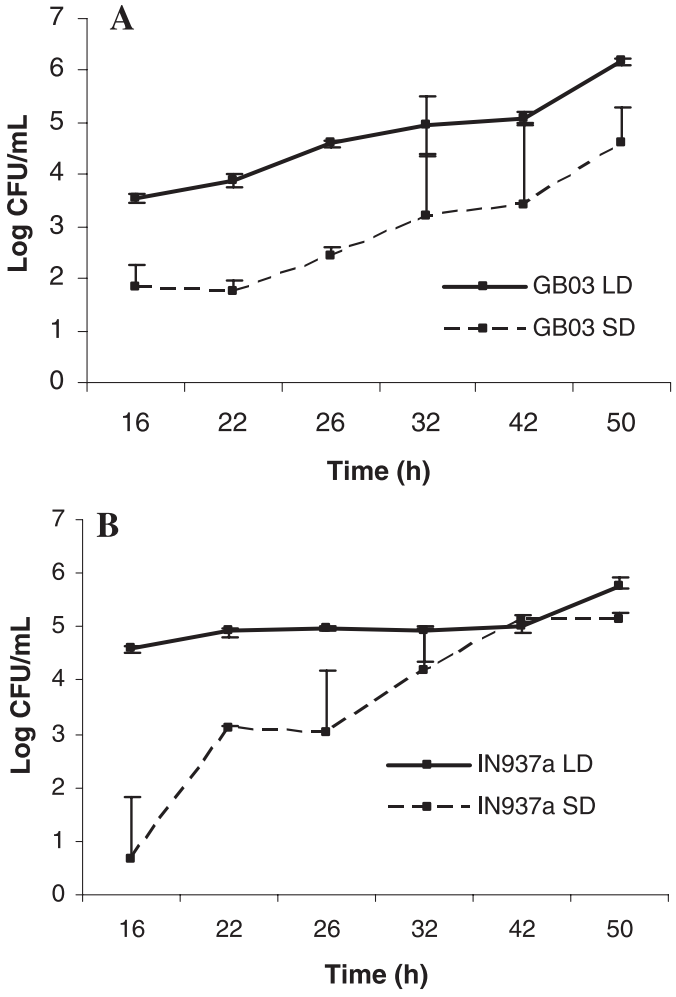
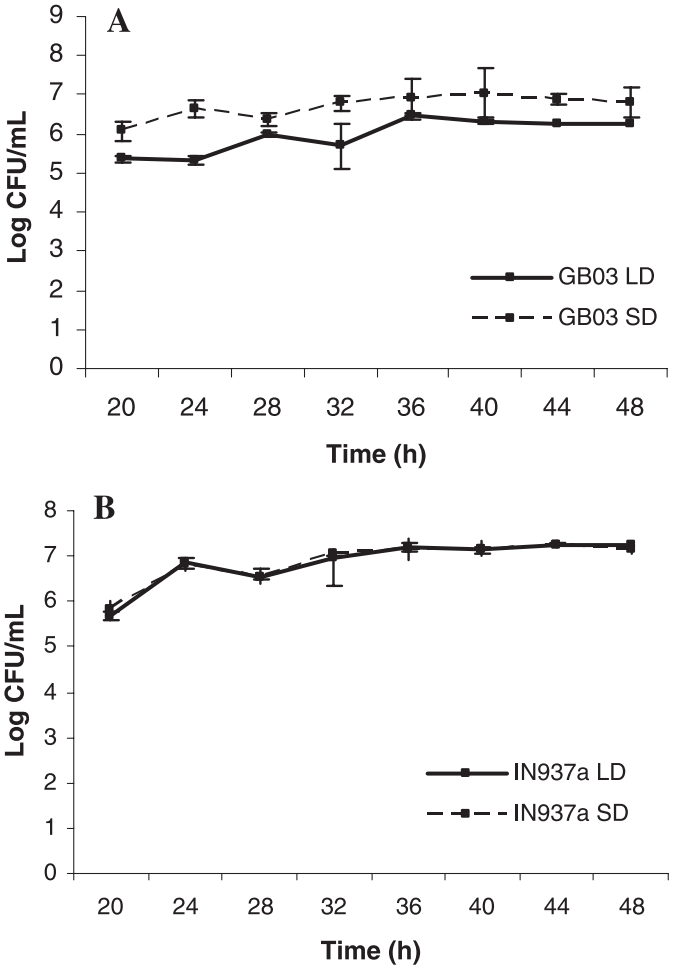


Fig. 2. Growth of strains GB03 (A) and IN937a (B) in leachates of tomato under long-day (LD) and short-day (SD) conditions. Error bars represent the standard deviation.



seedlings after 3 weeks under LD but not under SD conditions. With pepper under LD conditions (Table 1), treatment with the recommended rate of BioYield (1:100) resulted in increases ($P = 0.05$) of fresh and dry root and shoot masses, while under SD conditions, the same treatment reduced ($P = 0.05$) root fresh mass and had no effect on the other parameters. Under SD conditions, the application of a higher concentration of BioYield (1:40) resulted in increased fresh shoot mass only. On tomato (Table 1), treatment with both concentrations of BioYield resulted in increased ($P = 0.05$) fresh and dry masses of roots and shoots under LD conditions, while there were no effects on those parameters by BioYield under SD conditions.

Summer tests

Because the use of supplemental lights in the winter test increased air temperature by 2 °C, we conducted additional tests in the summer. In these tests, LD and SD plants were grown together on the same greenhouse bench, and SD plants were covered daily with boxes to achieve an 8 h photoperiod. Results were similar to the winter tests. On pepper (Table 2) under LD conditions, BioYield increased fresh and dry mass of roots and fresh mass of shoots, while no increases resulted with BioYield under SD conditions. On tomato (Table 2), both rates of BioYield increased root and shoot masses under LD but not under SD conditions. Elicitation of growth promotion with BioYield also occurred on marigold (Table 2) and cucumber (Table 2) under LD but not under SD conditions.

Influence of day length on changes in root architecture elicited by BioYield

The finding that the mass of plant root systems was typically increased by the PGPR product BioYield under LD conditions but not under SD conditions led us to investigate more details of root responses to the PGPR product. WinRhizo analysis of individual roots of pepper and tomato (Table 3) revealed that BioYield elicited more significant increases in various parameters under LD than under SD conditions. On pepper under LD conditions, BioYield at the rate of 1:100 (the label rate for pepper) elicited increases ($P = 0.05$) in root surface area, projected area, root volume, number of root tips, length of total roots, and length of the very fine roots (the two smallest diameter categories) (Table 3). In contrast, under SD conditions, the same treatment on pepper elicited an increase only in mean root diameter. The results on tomato (Table 3) were similar. Under LD conditions, BioYield at the rate of 1:40 (the label rate for tomato) elicited increases ($P = 0.05$) in root surface area, projected area, number of root tips, length of total roots, and length of the very fine roots (the two smallest diameter categories). However, under SD conditions, the same treatment rate on tomato elicited no increases in any of the eight measured parameters and decreased ($P = 0.05$) root volume.

Influence of day length on elicitation of ISR by PGPR in BioYield

BioYield elicited ISR against bacterial spot disease on both pepper and tomato grown under both SD and LD conditions (Table 4). Treatment with both rates of BioYield on both crops reduced ($P = 0.05$) the number of spots on upper and lower leaves under both day lengths. On tomato under SD conditions (Table 4), control plants had over 50% more lesions than under LD conditions; however, a similar increase in disease on control plants under SD conditions did not occur on pepper (Table 4).

Influence of day length on root colonization by PGPR

To determine if the observed differences in plant growth under LD and SD conditions were related to differential root colonization by the PGPR strains in BioYield, root colonization of tomato was monitored. Results (Table 5) indicate that strains *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a reached higher ($P = 0.05$) populations per gram of root at 1 week after planting under LD than under SD conditions. This increase continued at 2 weeks after planting for strain IN937a. At 3 weeks after planting, the populations of both strains were not different between plants under SD and LD conditions.

Quality of root leachates from plants under LD and SD conditions

To test the hypothesis that leachates of tomato and pepper under LD conditions were distinct from those under SD conditions, we calculated the growth rates of the two PGPR strains contained in BioYield in root leachates collected under both conditions. The results were very different with the two crops. On pepper (Fig. 1), leachates from LD conditions supported faster growth of both strains GB03 and IN937a over most of the sample period. Populations of GB03 in leachates from LD pepper were higher ($P = 0.05$) than populations in leachates from SD plants at four of the six sample periods (16, 22, 26, and 50 h) (Fig. 1A), and populations of IN937a in leachates from LD plants were higher than those in SD plants at the three earliest sampling times (16, 22, and 26 h) as well as in the last one (50 h) (Fig. 1B). In contrast, neither strain had higher populations at any sample time in leachates of tomato plants from LD than in SD conditions. In fact, strain GB03 had higher populations ($P = 0.05$) in leachates from SD than in those from LD conditions at five sample times (20, 24, 28, 32, and 44 h) (Fig. 2A). With strain IN937a, populations were not different ($P = 0.05$) at any sample time in leachates from plants grown under LD or SD conditions (Fig. 2B).

Discussion

The results reported here indicate that elicitation of plant growth promotion, but not ISR, by a model PGPR system is regulated by photoperiod. The finding that elicitation of ISR by BioYield was not regulated by day length was unexpected. Previous reports with *Bacillus* spp. PGPR that elicit ISR (reviewed in Kloepper et al. 2004*b*) indicated that with most strains, there is a relationship between growth promotion and ISR such that ISR usually does not occur in the absence of growth promotion. Our results demonstrate that under SD conditions, ISR is elicited by BioYield even though growth promotion is not. Hence, efforts to elucidate distinct signaling pathways leading to ISR and growth promotion by *Bacillus* spp. PGPR might be aided by examining

the biochemical and gene-activation steps of PGPR-treated plants grown under LD and SD conditions.

As shown in many different experiments, BioYield elicited plant growth promotion under LD but not under SD conditions. The initial tests in the winter used supplemental light to increase photoperiod, resulting in a slight increase in air temperature. Hence, changed temperature could be a confounding factor; however, this factor was removed in the summer tests. When plants were grown on the same bench in the summer, and day length was regulated by covering plants, growth promotion was again elicited only under LD conditions on pepper, tomato, cucumber, and marigold. On each crop under LD conditions, the primary effect of plant growth promotion was on root mass, which increased approximately 100% with BioYield-treatment (Table 2).

Although root mass is commonly used to indicate growth promotion by PGPR, root mass alone does not adequately describe many root functions involved in the plant–soil relationship (Costa et al. 2002). To overcome this limitation, many of the root characteristics that describe root architecture such as length, average diameter, surface area, and volume can be used to assess more completely the functional size of the root system. In this study, root architecture analyses confirmed and expanded the finding that growth promotion occurs under LD but not under SD conditions with BioYield. On both tomato and pepper, BioYield elicited significant increases in several root architecture parameters under LD but not under SD conditions (Table 3), including surface area, projected area, number of root tips, total root length, and length of smallest diameter classes of roots. It is interesting to note that such increases occurred on tomato with both rates of BioYield; however, on pepper, the increases occurred with the lower rate of BioYield (1:100), which is the label rate for pepper, but not at the higher rate (1:40), which is the label rate for tomato.

Changes in root architecture can profoundly affect the capacity of plants to absorb nutrients and water as well as the biotic interactions in the rhizosphere (López-Bucio et al. 2003). The enhancement of root architecture parameters by BioYield in both crops helps explain the observed growth promotion. Exhibited changes in morphology such as greater root length increase a plant's capacity to uptake water and solutes. Nutrient uptake depends on the total length and average diameter of roots (Zobel 2003). In our tests, increased root length was also associated with increased root surface area and projected area (the area occupied by roots). Increased root surface area may increase the exploratory capacity of the root system and influence uptake rates of nutrients. A large surface area is considered to be of key importance for nutrient acquisition (Marschener 1998).

The increase in root surface area may be explained by an increase in formation of root hairs and lateral roots. The surface of root hairs can represent up to 70% of the total root surface area (López-Bucio et al. 2003; Larcher et al. 2003). Root hairs are essential in root anchorage and for increasing the area of soil exploitable for the plant, and therefore, their major role is related to nutrient uptake (Gilroy and Jones 2000). In addition, root hairs define the number of root tips, which regulate the direction of root growth (Gilroy and Jones 2000), and are important in microbial–root interactions (Persello-Cartieaux et al. 2001). In this study, we used two diameter ranges (0–0.5 and 0.5–1.0 mm). These ranges represent the very fine roots that increased under LD conditions in both pepper and tomato. The increased length of these very fine roots may explain the overall plant mass increases observed in both crops, since fine roots (less than 1 mm in diameter) are generally thought to be active sites of nutrient uptake (Keith 1998; Zobel 2003).

One possible explanation for the reduced growth promotion under SD conditions is that the two PGPR strains in BioYield colonized roots less under SD than under LD conditions. Support for this explanation was obtained by the finding that PGPR strain IN937a had higher ($P = 0.05$) populations on roots of tomato at 1 and 2 weeks after planting under LD conditions (Table 5) and strain GB03 had higher populations on roots at 1 week after planting under LD conditions. Hence, both strains colonized roots on a per-gram basis during the first week after planting better under LD conditions. Because signal transduction events that ultimately lead to growth promotion are likely to begin at the earliest stages of seedling development, this increased early colonization under LD conditions could be a significant contributing factor to the observed growth promotion.

Another possible explanation for the overall lack of growth promotion under SD conditions is that the quality of root leachates was different, thereby resulting in slower growth rates of the PGPR strains in BioYield. Support for this explanation was obtained in leachates of pepper but not in those of tomato. With pepper, both strains GB03 and IN937a grew faster (Fig. 1) in leachates collected from plants grown under LD than under SD conditions. However, on tomato, strain GB03 grew faster on leachates from plants grown under SD conditions and strain IN937a grew at the same rate on leachates from plants grown under both conditions. Overall, these results indicate that the response of pepper and tomato root leachates to different day lengths depended on the plant. Altered leachates of pepper under LD conditions, resulting in faster bacterial growth, might partially explain why BioYield promoted growth under LD but not under SD conditions; however, this would not explain the differential growth promotion on tomato.

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