Inhibition of *Criconemella xenoplax* Egg Hatch by *Pseudomonas aureofaciens*

S. W. Westcott III and D. A. Kluepfel

Assistant and associate professors, respectively, Department of Plant Pathology and Physiology, 120 Long Hall, Box 340377, Clemson University, Clemson, SC 29634-0377.

Technical contribution 3358 of the South Carolina Agricultural Experiment Station, Clemson University. Funded in part by USDA-ARS Special Grant 91-34126-5914.

We thank Peter Burrows for advice on statistics and Stormy Ingold and Lynn Luszcz for technical assistance.

Accepted for publication 11 June 1993.

ABSTRACT

Westcott, S. W., III, and Kluepfel, D. A. 1993. Inhibition of *Criconemella xenoplax* egg hatch by *Pseudomonas aureofaciens*. Phytopathology 83:1245-1249.

Nine fluorescent pseudomonad strains and one strain each of *Escherichia coli* and *Rhizobium fredii* were tested for potential to inhibit *Criconemella xenoplax* egg hatch. One strain, *Pseudomonas aureofaciens* BG33, and its genetically modified derivative, BG33CL1R, effectively inhibited egg hatch at 2.0×10^8 cfu ml^{-1}. Strain BG33CL1R inhibited egg hatch by 5, 50, and 95% when present at 1.0×10^7, 4.8×10^7, and 2.4×10^8 cfu ml^{-1}, respectively. Eggs were sensitive to BG33CL1R from the time of deposition until second-stage juveniles were formed inside eggs 6.5 days later. In contrast, activity of second-stage juveniles and adults did not appear to be affected by incubation in BG33CL1R for up to 2 wk. Seven strains of *P. aureofaciens* and one strain each of *E. coli, P. chlororaphis*, and *R. fredii* did not inhibit egg hatch in similar tests. Therefore, general septic conditions apparently are not responsible for egg kill. The potential for *P. aureofaciens* BG33 and BG33CL1R to kill *C. xenoplax* eggs could explain in part their suppression of nematode population increase in greenhouse tests.

Additional keywords: bacterial antagonist, biocontrol, *Mesocriconema xenoplax*.

Ectoparasitic phytonematodes feed on and spend a majority of their life cycle in the rhizosphere of host plants. Consequently, microbial residents of the rhizosphere represent a potential reservoir of biological control agents capable of limiting nematode multiplication (16,21,35). Because fluorescent pseudomonads are the most numerous residents in the rhizosphere community and possess native biocontrol capabilities (9,10,12,13,32,39,42), members of this group have been examined for their potential to control plant-parasitic nematodes (4,18). Pseudomonads can colonize the rhizosphere after they are introduced into soil on seeds of crop plants (19,31,36,38). Thus, there is potential for discovery of new agents useful for biological control of nematodes among this group of bacteria.

It is commonly postulated that soils suppressive to plant-parasitic nematodes would be ideal sites for exploration of new biological control agents. Peach orchard sites suppressive of *Criconemella xenoplax* (Raski) Luc and Raski (=*Mesocriconema xenoplax* (Raski) Loof & de Grisse) have been discovered, characterized, and explored for fluorescent pseudomonads that affect nematode population growth (20). Seven strains of *Pseudomonas aureofaciens* affected nematode population growth in greenhouse tests. Selection of appropriate methods for use of these bacteria in biological control systems would be enhanced by knowledge of the mechanisms by which they limit nematode multiplication.

In preliminary experiments, one strain affected nematode egg hatch. Our objective has been to characterize this interaction and provide evidence that it involves a specific interaction between the bacteria and the nematode. An abstract of this work has been published (41).

MATERIALS AND METHODS

Bacteria. Bacterial species were determined by a statistical comparison of fatty acid retention time profiles (30) to those of known species in the Microbial Identification System trypticase soy broth agar database library version 3.3 (2). All strains were checked for growth and fluorescence on the selective medium S1 (14). Six strains (BG33, BG33CL1R, A1-10, A5-4, G2-6, and H1-7) had been identified as *P. aureofaciens* and were found to inhibit population increase in greenhouse tests (20). Strain BG33CL1R, derived from BG33 in our laboratory, is resistant to rifampicin and contains the lacZY::Tn7 construct, both of which facilitate tracking this strain in natural environments (20). Strains of *Escherichia coli* DH5a, and *Rhizobium fredii* USDA257 (3) from our laboratory culture collection were included as controls to substantiate the ability of nematodes eggs to hatch when immersed in bacterial suspensions.

Nematode cultures. Soil collections made at the Sandhill Research and Education Center near Elgin, SC, were the source of nematodes from which cultures of *C. xenoplax* were established in the greenhouse. Nematodes were cultured on seedlings of *Prunus persica* (L.) Batsch. 'Nemaguard' in sand and were periodically subcultured by transfer of extracted nematodes to 2-wk-old Nemaguard seedlings. Plants were fertilized biweekly with half-strength Hoagland's solution containing K_2NO_3 and $CaNO_3$ as the sources of nitrogen (15).

Egg hatch assay. *C. xenoplax* were extracted from soil by elutriation (11) combined with centrifugal flotation (17). Twelve to 20 gravid females were transferred by hand into distilled water (3 ml) in a 35-mm-diameter plastic petri dish and allowed to deposit eggs in the dark at 26 C for 24 h. After egg deposition, females were removed, and eggs were counted and treated by addition of bacterial suspensions or distilled water. Gravid females typically deposit two to four eggs per day over a 4-day period (40). Since each set of 12–20 females was preserved as a group during 3–4 days of egg collection, up to four experiments could be derived from a single set of gravid females.

During incubation of eggs in the dark at 26 C, evaporation from petri dishes was limited by enclosure of dishes in a plastic box that was loosely covered. Two replications, each consisting of a single dish containing 20–90 eggs, were used in each treatment. Under these conditions, eggs hatch in approximately 9 days (40). To be sure that all eggs had sufficient time to hatch, numbers of hatched second-stage juveniles were tallied 2 wk after deposition. Most experiments included 12–15 petri dishes with eggs divided among desired treatments. In all cases, treatments were randomly assigned to petri dishes for each experiment.

Bacterial screen. Cell suspensions of different strains and species of bacteria were added to nematode eggs to determine their ability to kill *C. xenoplax* eggs. *Pseudomonas* spp. were grown on Pseudomonas Agar F (PAF; Difco, Detroit, MI). *E. coli* was grown on Luria-Bertani agar (LBA), which contained 10 g of Bacto tryptone (Difco, Detroit, MI), 10 g of NaCl, 5 g of yeast extract, and 15 g of agar per liter of distilled water. *R. fredii* was grown on yeast-extract-mannitol agar (YEM) (37). Suspensions were prepared from bacteria grown on agar medium for 24 h at 26 C. Cells were scraped from the agar surface and suspended in sterile distilled water. Bacterial concentrations were standardized spectrophotometrically to approximately 2×10^8 cfu ml^{-1}. After bacterial application, bacterial concentrations were measured by serial dilution and plating on an appropriate medium. Bacterial concentrations greater than 3×10^7 cfu ml^{-1} were used in the screening experiments. Nematode eggs were treated with bacterial suspensions immediately after females were removed from petri dishes.

Most experiments included four bacterial strains plus the positive (BG33CL1R) and negative (water treatment) controls. The objective was to determine the ability of a variety of strains to kill nematode eggs at concentrations of 2×10^8 cfu ml^{-1}. The positive control was BG33CL1R because this strain killed eggs and because, if necessary, its population could be assessed at the end of an experiment. Each strain was included in at least three experiments. The proportion of eggs hatched was adjusted to a fraction of the hatch of respective water controls for analysis as described below.

Dose-response assays. Although both BG33 and BG33CL1R killed nematode eggs, BG33CL1R was selected for further characterization because this strain can be tracked in natural environments, which will be important in subsequent research. Experiments were designed to determine effective bacterial concentrations for egg kill. BG33CL1R was grown on PAF, and bacterial suspensions were prepared as described above. Five concentrations of BG33CL1R were included in each test. Each concentration was delivered into two petri dishes containing eggs. The final volume of liquid in petri dishes was adjusted to a uniform amount for each experiment. Final concentrations of the bacterial suspension in petri dishes were determined by dilution plating. Equivalent volumes of sterile distilled water were added to control dishes. Due to variation in bacterial concentration among tests, the experiment was repeated 20 times to fully characterize the transition between effective and ineffective concentrations (1.4×10^7 to 2.8×10^9 cfu ml^{-1}).

The results of these experiments were combined for analysis. A logistic model was fitted to results by maximum likelihood methods, with the proportion of eggs hatched weighted by the number of eggs per dish. The expected proportion of eggs that hatch for each dose, $P(\mathrm{d})$, was determined from the logistic model:

$$P(\mathrm{d}) = 1/\{1+[e^{\alpha - \beta(\ln d)}]\},$$

where e is the base of the natural logarithm, α and β are parameters in the logistic model, and ln d is the natural logarithm of dose (cfu ml^{-1}). The natural logarithm of the dose at which half of eggs are expected to hatch (γ) may be determined directly from the equation $\gamma = \alpha/\beta$.

For this analysis, counts for each pair of dishes that received the same treatment were combined as one observation. The harmonic mean of the number of eggs in each dish (N') was calculated as:

$$N' = 2/[(1/N) + (1/N_o)],$$

where N is the number of eggs deposited for each treatment and N_o is the mean number of eggs in the respective water controls. The respective water controls for this computation were those in the group of experiments that shared the same population of gravid females as the source of eggs. N' was used as the weighting factor for fitting the logistic model. To account for less than 100% hatch in the water controls, the adjusted number of juveniles hatched at each treatment dose (J') was determined using the complement of Abbott's correction for mortality (1) as:

$$J' = J/(J_o/N_o),$$

except for cases in which $J/N > J_o/N_o$, in which case

$$J' = N',$$

where J is the number of juveniles that hatched at each concentration, and J_o is the mean number of juveniles hatching in the respective water controls. The respective water controls contained eggs from the same set of gravid females that provided eggs for treatment dishes.

Duration of egg sensitivity. The objective of these tests was to determine the duration of sensitivity of eggs to bacteria during their development. Nematode eggs were treated with *P. aureofaciens* BG33CL1R at approximately 2.0×10^8 cfu ml^{-1} immediately after removal of females from petri dishes and at 1-day intervals for up to 7 days after eggs were deposited. Bacterial suspensions were prepared from 24-h cultures on PAF. Concentrations were standardized and added to eggs as described above.

For this test, eggs were collected from a single set of females distributed among 12 dishes (12–15 females per dish) on four

consecutive days. Bacterial suspensions were added to four dishes in each of the four sets of dishes on day four, immediately after the last set of eggs was collected. Four days later (day eight), bacteria were introduced into four additional dishes prepared for each of the 4 days during the collection period. Two dishes from each collection day were treated by addition of sterile distilled water on each inoculation date. This resulted in inoculation of eggs that were approximately 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, or 7.5 days old. The proportion of eggs hatched was determined 14 days after egg deposition. This experiment was repeated twice.

A similar experiment was conducted in a which females (50 per dish) were allowed to deposit eggs for only 5 h. Females were incubated at 26 C overnight between collections made during the day. The 5-h collection period produced clutches of eggs that were in similar stages of development. These eggs were photographed before and after bacterial treatment to determine the relationship between stage of development and sensitivity to BG33CL1R. The proportion of eggs that hatched was determined 2 wk after eggs were deposited.

TABLE 1. Proportion egg hatch by *Criconemella xenoplax* when eggs were treated with different strains of bacteria[a]

Strain	10^7 cfu[b]	N[c]	Hatch[d]
Pseudomonas aureofaciens			
BG33	3.6–26	20	0.01 ± 0.03
BG33CL1R	1.8–99	47	0.15 ± 0.32
A1-10	6.2–21	17	0.94 ± 0.14
A5-4	7.6–13	6	0.91 ± 0.12
BA5	6.8–11	6	0.97 ± 0.06
E2-7	11.0–80	17	0.83 ± 0.22
G2-2	11.0–62	17	0.84 ± 0.18
G2-6	7.3–69	12	0.77 ± 0.17
H1-7	9.0–11	6	0.78 ± 0.20
P. chlororaphis			
A1-3	8.4–51	17	0.85 ± 0.19
Escherichia coli			
DH5α	3.4–40	27	0.76 ± 0.25
Rhizobium fredii			
USDA257	5.7–47	6	0.93 ± 0.12

[a] Table compiles results from all screening experiments. Due to variations in bacterial concentrations among experiments, no direct comparisons among strains are warranted. However, results allow classification of each strain as to its ability to kill nematode eggs.
[b] Range of initial concentrations of bacteria in suspension around nematode eggs as determined by serial plate counts.
[c] Number of dishes of eggs included in average proportion hatched.
[d] Mean proportion of eggs hatched and standard deviation after 2 wk. Values have been adjusted to a fraction of control hatch in respective experiments.

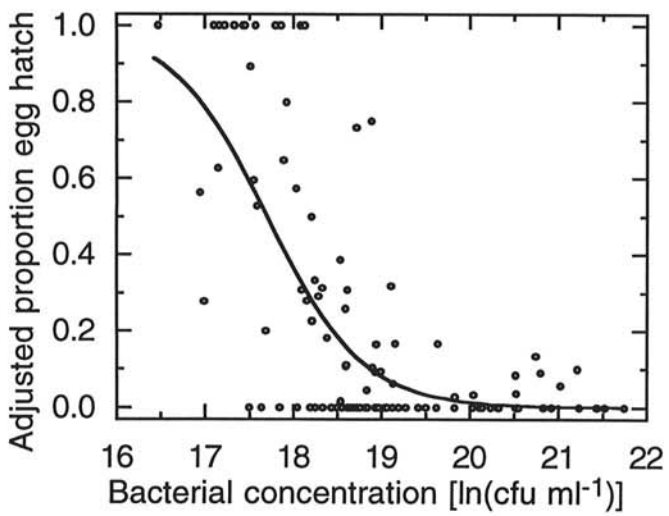


Fig. 1. Adjusted proportion of *Criconemella xenoplax* eggs that hatched in different bacterial concentrations of *Pseudomonas aureofaciens* BG33CL1R. The proportion of eggs hatching was adjusted with respect to water controls, and bacterial concentration was transformed by the natural logarithm of the colony-forming-units per milliliter (ln(cfu ml^{-1}). The plot represents the expected response based upon maximum likelihood methods.

RESULTS

Bacteria. All pseudomonad strains grew and fluoresced on the selective medium S1. Fatty acid analysis using the Microbial Identification System revealed strain A1-3 to be *P. chlororaphis* (similarity index [*I*] = 0.868), whereas strains E2-7 (I = 0.818), G2-2 (I = 0.717), and BA5 (I = 0.719) were shown to be *P. aureofaciens*.

Bacterial screen. Only BG33 and BG33CL1R inhibited egg hatch at the range of concentrations tested (Table 1). In tests that included both BG33 and BG33CL1R, both killed nematode eggs, and no differences in their behavior were discovered. In all other treatments receiving bacteria, however, egg hatch was delayed approximately 1 day, and the proportion of eggs hatched was slightly depressed compared with the proportion for water controls.

Dose-response assays. With the water control, 51 ± 24% of eggs hatched. The maximum likelihood estimates for the model parameters were −32.98 ± 0.15 for α and −1.86 ± 0.07 for β (Fig. 1). The median effective dose (γ) was estimated to be 17.7 ± 0.03, which indicates that a dose of BG33CL1R of 4.8×10^7 cfu ml^{-1} was expected to kill 50% of the eggs that would normally hatch. Estimates for dosages expected to kill 5 and 95% of eggs were 1.0×10^7 and 2.4×10^8 cfu ml^{-1}, respectively.

Duration of egg sensitivity. Eggs were sensitive to application of BG33CL1R up until the time when the second-stage juvenile had formed in the egg (Fig. 2). In water controls, formation of stylets, which indicates development of the second-stage juvenile, was first observed 6.5 days after egg deposit (Fig. 3). Egg development appeared to cease soon after application of bacteria. When bacteria were added soon after egg deposit, egg development ceased, and neither juvenile stage formed (Fig. 4). Some nematodes that were killed when bacteria were added late in their development (after 5.2 days) produced stylets before dying (Fig. 5). The internal integrity of most eggs exposed to effective concentrations of BG33CL1R was completely lost by the end of the 2-wk incubation. However, egg shells appeared to be uncompromised. Adults and hatched second-stage juveniles survived and continued to move in BG33CL1R suspensions for 2 wk.

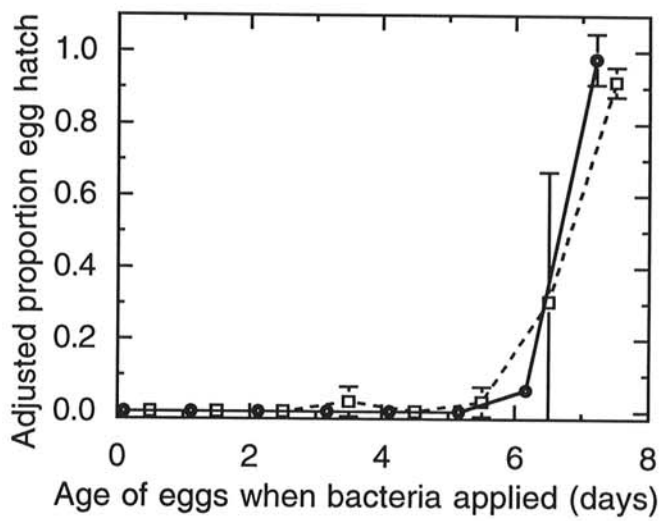
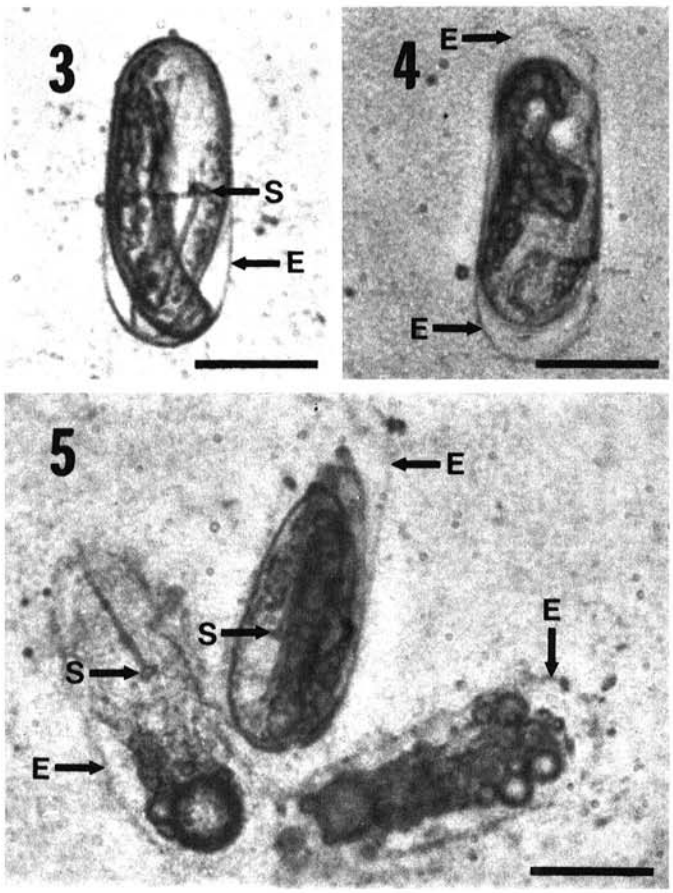


Fig. 2. Adjusted proportion of *Criconemella xenoplax* eggs of different ages that hatched in treatments of *Pseudomonas aureofaciens* BG33CL1R. The proportion of eggs hatching was adjusted to a fraction of water controls, and standard deviations are shown. Second-stage juveniles were first observed in eggs at 6.5 days. Open squares depict the response for eggs deposited over a 24-h period; therefore age varied by ± 12 h. Closed circles depict the response for eggs deposited over a 5-h period, with age varying by ± 2.5 h.

DISCUSSION

All fluorescent pseudomonads examined had been isolated or derived from bacteria isolated from peach roots collected from a soil suppressive to *C. xenoplax* and had affected nematode population growth in greenhouse studies (20). However, only BG33 and BG33CL1R inhibited egg hatch. All except one of these strains inhibited *C. xenoplax* population growth in the greenhouse. One strain, G2-2, stimulated nematode population increase (D. A. Kluepfel and T. M. McInnis, *unpublished data*). Suspensions of BG33 and BG33CL1R killed eggs in a reproducible manner not exhibited by the other strains tested; thus the lethal effect did not result from general septic conditions. All other bacteria included in these tests reduced hatch below that occurring in water controls (Table 1), but this activity was markedly different from the degree of egg kill seen with BG33 and BG33CL1R (usually 100%).

Bacteria may produce compounds toxic to nematodes as a result of cellular metabolism. For example, ammonia released by bacteria growing on nitrogen-rich media is toxic to most nematode species (29). This may be the mechanism of action for egg kill in vitro for BG33 and BG33CL1R and deserves further exploration. However, if this is the mechanism for egg kill, it seems unlikely that it would explain the activity of these bacteria against *C. xenoplax* in the greenhouse, where nitrogen compounds would not be available at such high concentrations.



Figs. 3–5. *Criconemella xenoplax* eggs incubated in distilled water with and without addition of bacteria. **3.** Seven-day-old *C. xenoplax* egg incubated in distilled water without addition of bacteria. The second-stage juvenile has formed. **4.** Seven-day-old *C. xenoplax* egg incubated in water for 4.1 days before addition of *Pseudomonas aureofaciens* BG33CL1R for the remainder of the incubation period. Development ceased soon after addition of bacteria, and none of the eggs had hatched by the end of the 2-wk incubation. **5.** Eight-day-old *C. xenoplax* egg incubated in water for 5.2 days before addition of *P. aureofaciens* BG33CL1R for the remainder of the incubation period. None of the eggs had hatched by the end of the 2-wk incubation. Appearance of a stylet indicated that molting to the second-stage juvenile had begun before death for some eggs. E = egg shell, S = stylet. Bars = 100 μm.

Development of eggs appeared to stop soon after bacterial treatment, suggesting rapid injury of treated eggs. Only stages of the nematode that developed before formation of the second-stage juvenile in the egg were sensitive to the bacteria. Since adults and second-stage juveniles were apparently insensitive, it appears that the nematode cuticle and not the egg shell is a selective barrier to the egg-kill factor. First-stage juveniles appeared to be sensitive, indicating that the cuticle of this stage may not be an effective barrier. Alternatively, first-stage nematodes may be sensitive to the bacteria during the first molt.

At least three general mechanisms of action for bacteria affect nematode egg hatch. First, some plant-parasitic nematodes require a hatching factor derived from the host plant, and some bacteria prevent this signal from being received by the nematode (24,25,28). This would not be an important mechanism for antagonism of egg hatch for *C. xenoplax*, since eggs of this nematode do not require stimulation from the plant host for hatching (34,40).

Second, eggs may be lysed by bacteria that produce various lipolitic, proteolytic, and chitinolytic enzymes (26). When living eggs were treated with chitinase, they became spherical and nematodes hatched prematurely (23). We saw no evidence of egg shell rupture or changes in the shape of eggs in our study. However, changes in permeability could occur without dramatic changes in egg shape, as has been documented for exotoxins produced by *Bacillus thuringiensis israelensis* (6). This potential mechanism of action requires further study for BG33CL1R.

The third mechanism involves production of toxins. *B. thuringiensis* appears to produce a number of exotoxins that kill eggs of a ruminant nematode and of *Meloidogyne* spp. (5,7,8, 22,27). A large number of known metabolites produced by fluorescent pseudomonads are toxic to other microorganisms (33). Some of these may be toxic to plant-parasitic nematodes (18), and a few may be toxic during specific stages of development. As yet, little is known about how these toxins cause injury to nematode eggs, and any involvement of toxins with egg kill by BG33 and BG33CL1R awaits proof.

Discovery of BG33 and development of its marked strain offer the opportunity to identify specific factors active against *C. xenoplax* eggs. If these factors prove to be unique compounds, they may be utilized in a number of ways in nematode management programs. The bacteria may be effective when applied to crop roots in the field, or the egg-kill factor gene(s) may be transferred into superior root-colonizing bacterial strains or into the plant itself. Further efforts are in progress to isolate and characterize the egg-kill factors produced by BG33 and BG33CL1R and to identify the genes controlling their activity.

LITERATURE CITED

1. Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265-267.
2. Anonymous. 1988. Microbial Identification System (MIS): Systems Users Manual. Microbial ID, Inc., Newark, DE.
3. Balatti, P. A., and Pueppke, S. G. 1990. Nodulation of soybean by a transposon-mutant of *Rhizobium fredii* USDA257 is subject to competitive nodulation blocking by other rhizobia. Plant Physiol. 94:1276-1281.
4. Becker, J. O., Zavaleta-Mejia, E., Colbert, S. F., Schroth, M. N., Weinhold, A. R., Hancock, J. G., and Van Gundy, S. D. 1988. Effects of rhizobacteria on root-knot nematodes and gall formation. Phytopathology 78:1466-1469.
5. Bone, L. W., Bottjer, K. P., and Gill, S. S. 1985. *Trichostrongylus colubriformis*: Egg lethality due to *Bacillus thuringiensis* crystal toxin. Exp. Parasitol. 60:314-322.
6. Bone, L. W., Bottjer, K. P., and Gill, S. S. 1987. Alteration of *Trichostrongylus colubriformis* egg permeability by *Bacillus thuringiensis israelensis* toxin. J. Parasitol. 73:295-299.
7. Bone, L. W., Bottjer, K. P., and Gill, S. S. 1988. Factors affecting the larvicidal activity of *Bacillus thuringiensis israelensis* toxin for *Trichostrongylus colubriformis* (Nematoda). J. Invertebr. Pathol. 52:102-107.
8. Bottjer, K. P., Bone, L. W., and Gill, S. S. 1985. Nematoda: Susceptibility of the egg to *Bacillus thuringiensis* toxins. Exp. Parasitol. 60:239-244.

9. Brisbane, P. G., and Rovira, A. D. 1988. Mechanisms of inhibition of *Gaeumannomyces graminis* var. *tritici* by fluorescent pseudomonads. Plant Pathol. 37:104-111.
10. Burr, T. J., and Caesar, A. 1984. Beneficial plant bacteria. Crit. Rev. Plant Sci. 2:1-20.
11. Byrd, D. W., Jr., Barker, K. R., Ferris, H., Nusbaum, C. J., Griffin, W. E., Small, R. H., and Stone, C. A. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. J. Nematol. 8:206-212.
12. Davison, J. 1988. Plant beneficial bacteria. Bio/Technology 6:282-286.
13. Fravel, D. R. 1988. Role of antibiosis in the biocontrol of plant diseases. Annu. Rev. Phytopathol. 26:75-91.
14. Gould, W. D., Hagedorn, C., Bardinelli, T. R., and Zablotowicz, R. M. 1985. New selective media for enumeration and recovery of fluorescent pseudomonads from various habitats. Appl. Environ. Microbiol. 49:28-32.
15. Hoagland, D. R., and Arnon, D. I. 1950. The water-culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347.
16. Jatala, P. 1986. Biological control of plant-parasitic nematodes. Annu. Rev. Phytopathol. 24:453-489.
17. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48:692.
18. Kloepper, J. W., Rodriguez-Kabana, R., McInroy, J. A., and Young, R. W. 1992. Rhizosphere bacteria antagonistic to soybean cyst (*Heterodera glycines*) and root-knot (*Meloidogyne incognita*) nematodes: Identification by fatty acid analysis and frequency of biological control activity. Plant Soil 139:75-84.
19. Kloepper, J. W., Schroth, M. N., and Miller, T. D. 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70:1078-1082.
20. Kluepfel, D. A., McInnis, T. M., and Zehr, E. I. 1993. Involvement of root-colonizing bacteria in peach orchard soils suppressive of the nematode, *Criconemella xenoplax*. Phytopathology 83:1240-1245.
21. Mankau, R. 1981. Microbial control of nematodes. Pages 475-494 in: Plant Parasitic Nematodes, Vol. III. B. M. Zuckerman and R. A. Rohde, eds. Academic Press, New York.
22. Meadows, J., Gill, S. S., and Bone, L. W. 1989. Factors influencing lethality of *Bacillus thuringiensis kurstaki* toxin for eggs and larvae of *Trichostrongylus colubriformis* (Nematoda). J. Parasitol. 75:191-194.
23. Mercer, C. F., Greenwood, D. R., and Grant, J. L. 1990. Effect of chitinase on *Meloidogyne hapla* eggs and larvae, a potential resistance factor? Nematologica 36:371.
24. Oostendorp, M., and Sikora, R. A. 1989. Seed treatment with antagonistic rhizobacteria for the suppression of *Heterodera schachtii* early root infection of sugar beet. Rev. Nematol. 12:77-83.
25. Oostendorp, M., and Sikora, R. A. 1990. In-vitro interrelationships between rhizosphere bacteria and *Heterodera schachtii*. Rev. Nematol. 13:269-274.
26. Parker, N., Hayward, A. C., and Stirling, G. R. 1988. Effect of chitinolytic soil bacteria on root-knot nematode eggs. Int. Congr. Plant Pathol., 5th, Abstracts of Papers. Kyoto, p. 157.
27. Prasad, S. S. V., Tilak, K. V. B. R., and Gollakota, K. G. 1972. Role of *Bacillus thuringiensis* var. *thuringiensis* on the larval survivability and egg hatching of *Meloidogyne* spp., the causative agent of root-knot disease. J. Invertebr. Pathol. 20:377-378.
28. Racke, J., and Sikora, R. A. 1992. Influence of the plant health-promoting rhizobacteria *Agrobacterium radiobacter* and *Bacillus sphaericus* on *Globodera pallida* root infection of potato and subsequent plant growth. J. Phytopathol. 134:198-208.
29. Rodriguez-Kabana, R. 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. J. Nematol. 18:129-135.
30. Sasser, M. 1990. Identification of bacteria through fatty acid analysis. Pages 199-204 in: Methods in Phytobacteriology. Z. Klement, K. Rudolph, and D. Sands, eds. Akademiai Kiado, Budapest.
31. Scher, F. M., Ziegle, J. S., and Kloepper, J. W. 1984. A method for assessing the root-colonizing capacity of bacteria on maize. Can. J. Microbiol. 30:151-157.
32. Schippers, B., Bakker, A. W., and Bakker, P. A. H. M. 1987. Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Annu. Rev. Phytopathol. 25:339-358.
33. Schippers, B., Lutenberg, B., and Weisbeek, P. J. 1987. Plant growth control by fluorescent pseudomonads. Pages 19-39 in: Innovative Approaches to Plant Disease Control. I. Chet, ed. J. Wiley & Sons, New York.
34. Seshadri, A. R. 1964. Investigations of the biology and life cycle of *Criconemoides xenoplax* Raski, 1952 (Nematoda: Criconematidae). Nematologica 10:540-562.
35. Stirling, G. R. 1991. Biological control of plant parasitic nematodes: Progress, problems, and prospects. CAB International, Wallingford, Oxon, UK.
36. Suslow, T. V., and Schroth, M. N. 1982. Rhizobacteria of sugar beets: Effects of seed application and root colonization on yield. Phytopathology 72:199-206.
37. Vincent, J. M. 1970. A manual for the practical study of root nodule bacteria. Blackwell Scientific Publications, Oxford, UK.
38. Weller, D. M. 1983. Colonization of wheat roots by a fluorescent pseudomonad suppressive to take-all. Phytopathology 73:1548-1553.
39. Weller, D. M. 1988. Biological control of soil-borne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. 26:379-407.
40. Westcott, S. W., and Burrows, P. M. 1991. Degree-day models for predicting egg hatch and population increase of *Criconemella xenoplax*. J. Nematol. 23:386-392.
41. Westcott, S. W., and Kluepfel, D. A. 1992. Inhibition of *Criconemella xenoplax* egg hatch by a strain of *Pseudomonas aureofaciens*. (Abstr.) J. Nematol. 24:626.
42. Windels, C. E., and Lindow, S. E., eds. 1985. Biological Control on the Phylloplane. American Phytopathological Society, St. Paul, MN.