Involvement of Root-Colonizing Bacteria in Peach Orchard Soils Suppressive of the Nematode *Criconemella xenoplax*

D. A. Kluepfel, T. M. McInnis, and E. I. Zehr

First and third authors, associate professor and professor, respectively, Department of Plant Pathology and Physiology, 120 Long Hall, Clemson University, Clemson, SC 29634-0377. Second author, associate professor, Department of Biological Sciences, Clemson University, Clemson, SC 29634-1903.

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ABSTRACT

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A field site suppressive to peach tree short life and the ring nematode *Criconemella xenoplax* was identified. Steam treatment of these soils eliminated suppressiveness, whereas small amounts of nonsteamed suppressive soil added to steamed soil inhibited *C. xenoplax* multiplication. Chemical and physical analysis of the soil detected no significant differences between suppressive and nonsuppressive soils, supporting the hypothesis that a biological agent(s) is important in *C. xenoplax* suppression. Among 290 randomly selected field strains of fluorescent pseudomonads from the rhizosphere of peach trees growing in suppressive soil, approximately 2.4% suppressed nematode population increases, 5.2% increased multiplication rates, and 92.4% had no effect in greenhouse bioassays. In root colonization tests, a suppressive strain exhibited the population dynamics typical for many root-colonizing pseudomonads. Bacterial populations increased to approximately 2×10^6 cells per gram of root and then declined to 5×10^2 cells per gram of root 12 wk later. Concomitantly, ring nematode population increases were significantly attenuated on peach seedlings receiving the bacterial treatment. These results suggest that rhizobacteria contribute to the antagonism of *C. xenoplax* in the suppressive soils.

The ring nematode *Criconemella xenoplax* Raski (Luc and Raski) (=Mesocriconema xenoplax (Raski) Loof and de Grisse) severely injures fine roots of peach (*Prunus persica* (L.) Batsch). This injury often is followed by premature mortality, particularly on replant sites in the southeastern United States (25,30). Tree mortality associated with ring nematodes is described as peach tree short life (PTSL) in the Southeast and as bacterial canker in California. In South Carolina, tree losses have nearly tripled since 1979, when the most effective nematicide, 1,2-dibromo-3-chloropropane (DBCP) was removed from the market. An average of 86,000 trees were lost annually between 1980 and 1992, at an estimated cost of 85 million dollars, due to PTSL (24). These heavy losses have prompted an intensive search for effective alternatives to DBCP for nematode control.

Alternatives to chemical nematicides to control the ring nematode and reduce the incidence of PTSL have included the use of nematode parasites (9), predators, antagonistic ground covers (23), and resistant cultivars (8,39). Organic and inorganic soil amendments, and the exploitation of naturally suppressive soils also have been used to control the multiplication of a variety of plant-parasitic nematodes (14,23,32).

Since most of the life cycle of *C. xenoplax* is spent at or near the root surface, microbes that readily colonize the rhizosphere (e.g., rhizobacteria) would be likely candidates for biological control agents. Although rhizobacteria have been proposed as biocontrol agents for root pathogens since the mid-1970s (33), investigators only recently have examined their potential to control phytoparasitic nematodes (20,28,40). Root-colonizing bacteria applied to seed or seed pieces at planting inhibited early root penetration of sugar beet and potato by *Heterodera schachtii* and *Globodera pallida,* respectively, in both greenhouse and field trials (26,28). Bacterial soil drenches suppressed *Meloidogyne incognita* galling of tomato, cucumber, and clover in greenhouse tests (6). Similar results were reported in greenhouse tests on cotton, tomato, peanut, and sugar beet treated with *Bacillus subtilis* to control *M. incognita, M. arenaria*, and *Rotylenchulus reniformis* (34). Plant species suppressive to nematode multiplication also have been shown to support a rhizosphere microflora enriched with species antagonistic toward phytopathogenic nematodes (19).

Soils naturally suppressive of nematode multiplication have been identified for several phytoparasitic nematode species (14,15, 35,41). To our knowledge, however, no soils have been identified as being suppressive to ectoparasitic nematodes such as *C. xenoplax*. Identification of field sites naturally suppressive to *C. xenoplax* provides an opportunity to identify potential biological control agents of this and other plant-parasitic nematodes. The natural antagonistic activity of these field sites toward ring nematode multiplication and the association of root-colonizing pseudomonads with this suppression are described here. Preliminary reports of this work have been published (18,22).

MATERIALS AND METHODS

PTSL incidence and field populations of C. xenoplax. The field site is located at the Clemson University Sandhill Research and Education Center, Elgin, SC. The original orchard was planted in February 1976 and contained four blocks (64 trees per block) of Redhaven and Loring scions grafted onto either Nemaguard, Lovell, 152 A1-2 Clayton, NA-8 Tennessee selection, Harrow 421, Siberian C, or Halford rootstocks in nonfumigated soil. In May 1982, the trees were rated, and percent mortality due to PTSL was calculated.

After initial random sampling throughout the orchard to determine ring nematode populations, two sub-blocks (sites) were selected for continuous monitoring. The two sites, one suppressive (<20 nematodes per 100 cm^3 of soil) and one nonsuppressive (>50 nematodes per 100 cm^3 of soil) to *C. xenoplax* multiplication, were assayed from June 1988 to October 1991. Each site contained 20 trees. Four soil subsamples per tree were collected from around the tree drip line, using a stainless steel sampling cone (15 cm deep). Subsamples were combined and gently mixed in a plastic

bag. A 100-cm^3 soil sample was removed, and nematodes were extracted by semiautomatic elutriation (7) followed by centrifugation-flotation (12).

Soil analysis. Samples of suppressive and nonsuppressive soils were assayed for the presence of 23 purgeable halocarbons, five aromatic residues, and 26 herbicides by The University of Georgia Cooperative Extension Service (2). Soil samples were extracted and analyzed using EPA gas-liquid chromatographic (GLC) methods 8010 and 8020 for detection and quantification of halocarbons and aromatic residues, respectively (2). Samples were analyzed for herbicide content by Soxhlet extraction of 30 g of soil overnight with ethyl acetate. Extracts were concentrated on a rotary evaporator and adjusted to a 10-ml volume with ethyl acetate-toluene (75:25) for cleanup by gel permeation chromatography (13). Samples were applied to a 2.5- × 27-cm column containing SX-3 Bio-Beads (3% divinylbenzene-polystyrene) and eluted with ethyl acetate-toluene (3:1, v/v). Analyses by gel permeation chromatography were done as described previously (13). The herbicide-containing fraction was collected in a 250-ml boiling flask and reduced to near dryness on a vacuum rotary evaporator. The extracts were made to a final volume of 2 ml of ethylacetate for GLC analysis.

Gas chromatographic analyses were conducted using a Tracor model 560 GLC unit equipped with a Tracor model 702N/P detector. GLC conditions were: SPB-5, glass capillary column (60 m × 0.75 mm i.d., 1.0 μm film, Supelco, Inc., Bellefonte, PA); carrier gas: helium with an approximate flow rate of 15 ml/min. The oven temperature program consisted of an initial temperature of 134 C followed by an increase of 10°/min to a final temperature of 275 C, with a final 5-min hold at 275 C in the detector. The inlet port temperature was 270 C. Herbicide levels were quantified by comparison of sample peak heights with known analytical standards (Supelco Inc.). A reagent blank and samples to which known quantities of herbicide (equivalent to 0.25 ppm) had been added were included with each analysis. Purgeable halocarbons assayed included vinyl chloride, 1,1-dichloroethylene, methylene chloride, *trans*-1,2-dichloroethylene, 1,1-dichloroethane, chloroform, trichloroethane, carbon tetrachloride, 1,2-dichloroethane, trichloroethylene, 1,2-dichloropropane, bromodichloromethane, *trans*-1,2-dichloropropane, *cis*-1,2-dichloropropene, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethylene, dibromochloromethane, chlorobenzene, bromoform, 1,1,2,2-tetrachloroethane, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,2-dichlorobenzene. Herbicides (common names) assayed included alachlor, atrazine, benefin, bromacil, butylate, chlorothalonil, cycloate, dimethoate, EPTC, hexazionone, isopropalin, metolachlor, metribuzin, molinate, napropamide, oxadiazon, oxyfluorfen, pebulate, pendimethalin, profluralin, propachlor, propazine, simazine, terbacil, trifluralin, and vernam. Aromatic residues assayed were benzene, toluene, ethyl benzene, and *o*-, *m*-, and *p*-xylene.

Inorganic elemental and organic matter analyses were performed by the Clemson University Agricultural Services Laboratory as previously described (1). Concentrations of P, K, Ca, Mg, and Al were measured.

Peach seedling preparation for greenhouse assays. Peach (cv. Nemaguard) seeds were soaked overnight in distilled water at 6 C, disinfested in 100% household bleach (5.25% sodium hypochlorite) for 3 min, and rinsed three times in sterile distilled water. Disinfested seeds were sandwiched between sterile paper towels moistened with a 0.025% slurry of captan 50W and stored at 4 C for 8–12 wk to induce germination. Germinating seeds were planted in trays of steamed (60–70 C for 4 h) river bottom sand and grown at 28 C under a light cycle of 16 h light, 8 h dark for 1–3 wk.

All plants were watered daily and fertilized biweekly. Plants grown in sand were fertilized with half-strength Hoagland's solution (11), whereas those grown in soil from the field sites were fertilized as described by Eayre et al (9). Pesticides (wettable sulfur, Safer insecticidal soap, Avid miticide) were applied as needed at the manufacturers' suggested rates.

Greenhouse assay of suppressive and nonsuppressive soil. Soil from the *C. xenoplax*-suppressive field site was collected and used within 48 h or stored in plastic bags at 10 C. The effect of soil steaming on nematode multiplication was assessed by planting 2-wk-old peach seedlings in plastic pots (500-cm^3 capacity) containing suppressive soil that was either nonsteamed, steamed (aerated steam for 1 h at 60–70 C), or a mixture of 5% nonsteamed and 95% steamed soil. Treatments were replicated 10 times. One to 2 wk after planting, seedlings were inoculated with adults and juveniles of *C. xenoplax* harvested from peach seedlings in pot cultures. Thirty nematodes suspended in tap water were added per 100 cm^3 of soil in small holes made in the soil adjacent to the base of the seedling. Fourteen to 16 wk later, nematodes were elutriated from the roots and soil as described above. Analysis of variance and Duncan's multiple range test ($P = 0.05$) were used to determine statistical differences between control and treatment means. The experiment was performed twice.

To compare nematode multiplication in suppressive and nonsuppressive soils, peach seedlings inoculated with *C. xenoplax* as described above were grown in both raw and steamed, suppressive and nonsuppressive soils. Fourteen to 16 wk after introduction, the nematodes were extracted and counted as described above. The treatments were arranged in a random design and replicated 10 times. The experiment was performed twice.

Bacterial isolation and greenhouse screening. Roots and soil from peach trees in the field site suppressive to nematode reproduction were collected from 2- to 15-cm depths. Small segments of feeder roots were separated from the soil, placed in sterile distilled water and shaken at 250 rpm for 15 min. Wash water was serially diluted in sterile distilled water and plated onto either Kings Medium B (16) amended with cycloheximide (100 μg/ml), or S1, a medium selective for fluorescent pseudomonads (10). One or 2 days later, discrete colonies were selected, purified, and then transferred to fresh media in culture tubes. For storage, cultures were transferred to microfuge tubes containing liquid Pseudomonas Agar F (PAF, Difco Laboratories, Detroit, MI) medium in 20% glycerol and placed in a −70 C freezer.

Purified bacterial strains were grown in 50 ml of liquid PAF (PAF minus the agar) medium overnight (200 rpm at 26 C). Bacteria were pelleted (8,000 × g for 10 min), resuspended in distilled water, and adjusted to a final concentration of 10^8 cfu/ml. Peach seedlings grown as described above were removed from the sand; roots were washed in sterile distilled water and then held in the bacterial suspension for 15–20 min. After inoculation, seedlings were planted individually in either 150-cm^3 nursery cones (3.8 × 20 cm) or 500-cm^3 plastic pots containing steamed nonsuppressive field soil. Ten milliliters of additional bacterial inoculum then was pipetted onto the soil surrounding each seedling. Controls consisted of seedlings to which only water and no bacteria were applied. Pots or cones were placed in the greenhouse. One to 2 wk later, seedlings were inoculated with *C. xenoplax* (30–80 nematodes per 100 cm^3 of soil) as described above. Nematodes were elutriated and counted 14–16 wk later. Based upon the degree-day model developed by Westcott and Burrows (37), 14–16 wk is the time required for a 10-fold increase in nematode population in water-treated controls.

Typically, 10–20 strains plus water-treated controls were examined during a preliminary bioassay, in which each bacterial strain was replicated four times (*data not shown*). All strains that inhibited nematode multiplication by 50% or more compared to the corresponding water controls were rescreened using four replicates per strain. Once again, all strains that inhibited multiplication by 50% or more were rescreened using 10 or 30 replicates per strain. All strains that suppressed nematode multiplication by 50% or more in at least two screenings were labeled suppressive strains and identified to species. Treatments were arranged in the greenhouse in a completely randomized design. Statistical significance between control and treatment means was determined by *t*-test.

Bacterial identification and genetic manipulation. Nematode-suppressive bacterial strains were identified using methods of fatty acid extraction and analysis described previously (3,31). In this procedure, fatty acids are extracted from the bacteria, saponified,

methyl esterified, and then analyzed using a Hewlett-Packard gas chromatograph. Retention time profiles were compared with the Microbial Identification System trypticase soy broth agar database version 3.3 (3,31). The species in the database with which they shared the highest similarity index were noted.

To facilitate unambiguous recovery in these and future laboratory and field experiments, the lacZY::Tn7 construct was inserted into the chromosome of *Pseudomonas aureofaciens* BG33, using previously described techniques and vectors (5). A spontaneous rifampicin-resistant derivative of the lacZY-engineered strain, *P. aureofaciens* BG33CL1, was selected and designated strain BG33CL1R. The lacZY::Tn7 construct facilitates utilization of lactose as a soil carbon source and cleavage of the chromogenic dye 5-bromo-4-chloro-3-indole β-D-galactopyranoside (X-gal) (Sigma, St. Louis, MO), both of which are traits not exhibited by wild-type fluorescent pseudomonads.

Nematode and bacterial population dynamics. Using the procedures described above, 2-wk-old peach seedlings were inoculated with *P. aureofaciens* BG33CL1R, ring nematodes, or both; planted in nursery cones containing steamed, nonsuppressive field soil; and grown in the greenhouse. Three treatments, BG33CL1R alone, BG33CL1R plus nematodes, and nematodes alone were sampled at weekly intervals for the first month and at biweekly intervals for the next 2 mo. *C. xenoplax* was introduced at a rate of 200 nematodes (adults and juveniles) per seedling 1 wk after planting. The plants were arranged randomly in a single block.

To estimate bacterial populations, four plants per treatment (BG33CL1R alone and BG33CL1R plus nematodes) were sampled. Soil was gently shaken from the roots, which were then weighed. Roots were ground in sterile distilled water, using a mortar and pestle on ice. The homogenate was serially diluted and plated onto PAF amended with cycloheximide (100 μg/ml), X-gal (40 μg/ml), and rifampicin (100 μg/ml). Following a 2-day incubation at 28 C, rifampicin-resistant blue colonies were tallied, and the number of cfu/g of fresh root weight was determined.

Nematode populations were estimated on each of four trees 4, 10, and 12 wk postplanting. To obtain nematode population estimates on individual plants, the entire contents of each cone, i.e., roots and soil, were suspended in two to three volumes (~ 400 ml) of distilled water and mixed with a fan blade stirrer for 30 sec. This mixture was allowed to settle for 15 sec and poured through a 35-mesh screen (500 μm). The eluant was poured through a 400-mesh screen (38 μm) from which the nematodes were collected. The eluant passing through the 400-mesh screen was added to the original soil-root mixture, mixed thoroughly, and the entire extraction procedure repeated twice. Nematodes collected from each of the three extractions were pooled and counted. This experiment was repeated once.

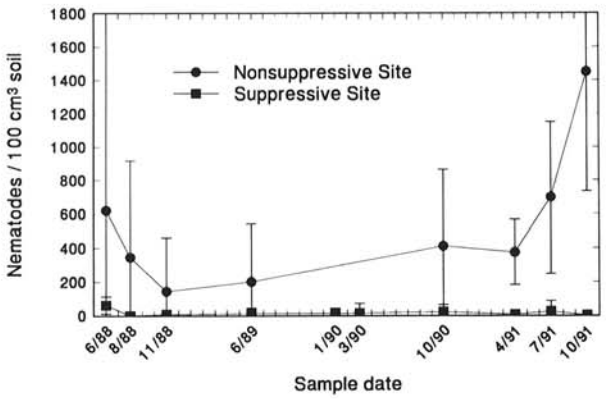

Fig. 1. *Criconemella xenoplax* populations from suppressive and nonsuppressive field sites from June 1988 to October 1991. Each data point represents the mean of 20 trees. Vertical bars represent ±1 standard deviation.

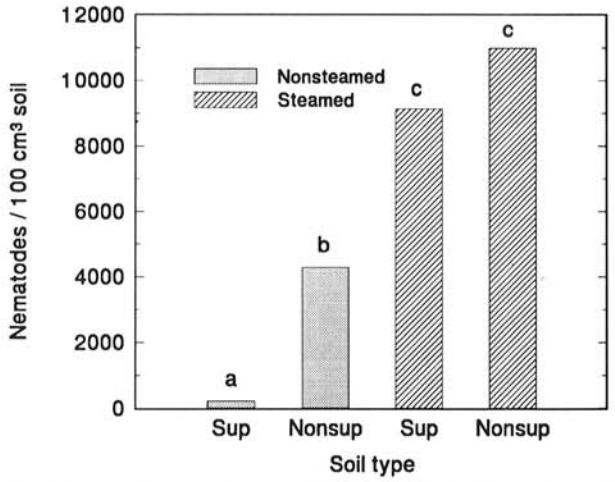

Fig. 2. *Criconemella xenoplax* populations on 14-wk-old peach seedlings grown in nonsteamed or steamed suppressive (Sup) and nonsuppressive (Nonsup) soil. Initial nematode density was 30 nematodes per 100 cm^3 of soil. Means separation based on Duncan's multiple range test ($P = 0.05$). Columns (means) with the same letter are not significantly different.

RESULTS

PTSL incidence and field populations of *C. xenoplax*. The first example of suppressiveness was observed in a portion of a replanted peach orchard at the Clemson University Sandhill Research and Education Center near Elgin, SC. Among the 64 trees planted in each of the four blocks, 30% in block 1, 17% in block 2, 28% in block 3, and 5% in block 4 died 6 yr after planting. Block 4 contains the site identified as suppressive of ring nematodes and PTSL.

Field populations of the ring nematode, over a 3-yr period, were consistently lower in the suppressive site than populations found in the adjacent site that was not suppressive (Fig. 1). In the nonsuppressive soil, nematode populations averaged 530 ± 416 nematodes per 100 cm^3 of soil over a 3-yr period from 1988 to 1991, whereas soil populations in the suppressive site averaged 16 ± 18 nematodes per 100 cm^3 of soil over the same period. On several of the 20 trees in the suppressive site, *C. xenoplax* was never detected.

Soil analysis. Both the suppressive and nonsuppressive soils were classified as Lakeland sand, composed of more than 90% sand and <5% organic matter. Organic residue analysis of soil extracts revealed no qualitative or quantitative differences between the two soils. Micro- and macroelement analysis of these two soils found aluminum levels to be significantly elevated in suppressive soil as compared to nonsuppressive soil (14.9 ± 8.3 vs. 4.1 ± 1.6 ppm, respectively). The pH levels in both soils were similar and ranged from 5.9 to 6.4.

Greenhouse assay of suppressive and nonsuppressive soil. Seedlings grown in suppressive soil supported significantly lower population densities of *C. xenoplax* than those in nonsuppressive soil (Fig. 2). Nematode populations increased significantly in both soils after steam treatment (Fig. 2). Nematode populations after 12 wk on peach seedlings in steamed suppressive soil were at least 10 times higher than those in nontreated suppressive soil. Seedlings in a mixture of 95% steamed:5% nontreated suppressive soil supported significantly fewer ring nematodes than those in

TABLE 1. Removal of suppression of *Criconemella xenoplax* populations on Nemaguard peach seedlings by steaming suppressive soil[a]

Soil treatment	*C. xenoplax* in 100 cm^3 of soil	
	Experiment 1	Experiment 2
Nonsteamed	75 ± 56 x[b]	18 ± 14 x
5% nonsteamed:95% steamed	296 ± 119 y	93 ± 58 x
Steamed	866 ± 428 z	196 ± 157 y

[a]60 C for 1 hr.

[b]Values within columns followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

TABLE 2. Bacterial strains that significantly reduced *Criconemella xenoplax* multiplication on Nemaguard peach seedlings planted in steamed, nonsuppressive field soil

Bacterial strain	Treatment	*C. xenoplax* in 100 cm³ of soil		
		First screen ($n = 4$ or 10)	Second screen ($n = 10$)	Third screen ($n = 30$)
BG-33	Inoculated	244 ± 77**[a]	8,104 ± 2,165	4,294 ± 2,554**
	Control	1,073 ± 153	9,468 ± 2,978	9,900 ± 8,462
A5-4	Inoculated	84 ± 2.0**	867 ± 566**	5,335 ± 4,441**
	Control	156 ± 57	2,578 ± 1,425	9,900 ± 8,462
G2-6	Inoculated	1,517 ± 1,445**	3,335 ± 5,907**	
	Control	7,596 ± 4,740	9,468 ± 2,978	Not tested
H1-7	Inoculated	4,832 ± 4,743**	992 ± 682**	
	Control	10,266 ± 4,080	2,578 ± 1,425	Not tested
A1-10	Inoculated	424 ± 162**	764 ± 605**	
	Control	923 ± 485	2,578 ± 1,425	Not tested
E3-17	Inoculated	4,940 ± 2,551[b]*	506 ± 2,578**	
	Control	10,104 ± 2,880	2,578 ± 1,425	Not tested
A3-17	Inoculated	4,672 ± 3,779*	1,132 ± 1,490**	
	Control	10,104 ± 2,880	2,578 ± 1,425	Not tested

[a] * and ** = difference in means significant at $P = 0.1$ or 0.05, respectively, as determined by t test.
[b] Four replications only.

steamed suppressive soil, but the reduction was less than in nontreated soil (Table 1). No parasitized *C. xenoplax* were observed in the suppressive soil, but the nematophagous fungus, *Hirsutella rhossiliensis,* was found in nematodes from nonsuppressive soil.

Bacterial isolation and bioassay. A total of 290 pseudomonad strains isolated from the rhizosphere of peach trees in the suppressive site were tested for nematode suppression. Seven strains (2.4%) suppressed nematode multiplication by more than 50% relative to the noninoculated controls in two or more independent greenhouse screenings (Table 2). Fifteen strains (5.2%) enhanced nematode multiplication twofold or more relative to noninoculated controls, and 92.4% of the strains tested had no effect on *C. xenoplax* multiplication.

Bacterial identification. The seven strains found to be antagonistic to ring nematode multiplication were all identified as *P. aureofaciens* (Table 2) using the Microbial Identification System (3). The similarity index of each strain to the database is shown in parenthesis after each strain: BG33 (0.840), A5-4 (0.413), G2-6 (0.919), A3-17 (0.813), H1-7 (0.837), A1-10 (0.100), and E3-17 (0.100).

Nematode and bacterial population dynamics. No differences were observed between the parental strain, *P. aureofaciens* BG33 and the derived strain BG33CL1R in ring nematode suppression in greenhouse bioassays (*data not shown*). This validated the use of BG33CL1R in these population dynamics studies.

Immediately after dip inoculation and planting, populations of *P. aureofaciens* BG33CL1R were approximately 6×10^5 cfu/g of root. Twenty-four hours later, populations decreased to a mean of 2×10^6 cfu/g of fresh root weight and then declined steadily to approximately 2×10^2 cfu/g of root 12 wk later (Fig. 3), regardless of the presence or absence of *C. xenoplax*. Only rifampicin-resistant colonies that turned blue in the presence of X-Gal were tallied. Regression analysis revealed no significant difference ($P = 0.56$) in the slopes of the two bacterial population curves (bacteria alone and bacteria plus nematodes). Although Figure 3 represents the results of one of two experiments, the second experiment gave nearly identical results.

Nematode populations were significantly suppressed in the presence of *P. aureofaciens* BG33CL1R on days 70 and 84 (Fig. 3). However, differences were not significant ($P = 0.05$) on all days, as determined by pairwise t-tests for each sample time.

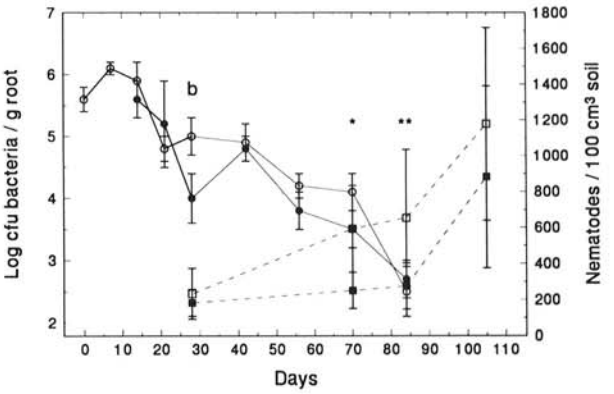

Fig. 3. Population dynamics of *Pseudomonas aureofaciens* BG33CL1R and *Criconemella xenoplax* on roots of peach seedlings. Seedling roots were inoculated with BG33CL1R by dipping in a suspension of 1×10^8 cfu/ml immediately before planting in cones containing steamed, nonsuppressive field soil. Seedlings were inoculated with *C. xenoplax* 1 wk postplanting at an initial density of 200 nematodes per 100 cm³ of soil. ○ = BG33CL1R populations in the absence of *C. xenoplax*, ● = BG33CL1R populations in the presence of *C. xenoplax*, □ = *C. xenoplax* populations in the presence of BG33CL1R, ■ = *C. xenoplax* populations in the absence of BG33CL1R. Each data point is the mean of four replications. Vertical bars represent ±1 standard deviation. Bacterial populations on the day labeled "b" were significantly different ($P = 0.05$). *C. xenoplax* populations on the days labeled * and ** were significantly different ($P = 0.05$ and 0.10, respectively).

DISCUSSION

Identification of a field site naturally suppressive to *C. xenoplax* multiplication is an important first step in the search for an effective biological control agent for this plant-parasitic nematode. The suppressiveness of this soil was confirmed in greenhouse tests, where soil from the site suppressed populations of *C. xenoplax* in the rhizosphere of peach seedlings (Table 1 and Fig. 2). The nearly identical edaphic factors of the suppressive and nonsuppressive sites and the absence of any detectable chemical residues in the suppressive sites indicated that biological agents were responsible for the observed suppression. Although Al concentrations were elevated in the suppressive site, no detectable effect on tree health or nematode infestation was detected in studies of soil aluminum by other researchers (C. Graham, *personal communication*). Steam treatment of the suppressive soil removed its antagonistic activity toward ring nematode multiplication (Table 1), a result consistent with biological activity. This hypothesis was further supported by the ability to restore suppression to pasteurized soil by adding a small amount of nontreated suppressive soil (Table 1). The fact that steaming also increased nematode numbers in nonsuppressive soil demonstrates that significant nonspecific antagonism probably exists in other soils also.

Seven of the 290 rhizosphere bacterial strains screened in the greenhouse were antagonistic toward *C. xenoplax* multiplication.

This rate of recovery of antagonistic rhizobacteria is comparable to that reported by others (26,27,40). The observation that some strains promoted nematode population increases also has been reported previously (26) and suggests that the observed biological control is unique to specific rhizobacterial strains.

Although several bacterial strains significantly reduced nematode multiplication in greenhouse assays, we rarely observed final nematode numbers below the damage threshold for PTSL of 38–83 nematodes per 100 cm^3 of soil (29). The reported threshold was estimated from field data, however, and may not be meaningful in greenhouse assays, where conditions are optimal for nematode multiplication. The question of whether rhizobacteria can prevent peach tree damage from nematodes must be answered in field trials.

The degree of nematode suppression may be correlated with the ability of the bacteria to inhabit the rhizosphere of inoculated plants. Oostendorp and Sikora (26) found that a *Pseudomonas fluorescens* strain applied to sugar beet seeds effectively colonized roots but rapidly declined during the first 15 days after inoculation to approximately 10^4 cfu/mm of root, a general trend reported by others (17,21,36). The colonization level was highly variable along the root and between plants, and the relationship of root colonization density and distribution to nematode antagonism was not reported.

When BG33CL1R was applied to peach roots, the bacterial populations declined from an initial population of approximately 2×10^6 cfu/g of root. *C. xenoplax* populations were reduced on those seedlings inoculated with this strain even though bacterial densities declined to less than 10^3 cfu/g of root (Fig. 3). Apparently, continuously high bacterial populations over the entire root system are not essential for nematode suppression, and BG33CL1R population dynamics are independent of the presence of nematodes.

The use of rhizobacteria as biocontrol agents has several potential advantages over the use of nematode parasites and predators. Important is the apparent absence of a strong density-dependent relationship between the rhizobacterium antagonist and the nematode, as is observed for parasites and predators. If the bacteria parasitized nematodes, one would expect to see an increase in bacterial populations in the presence of the host nematode. The absence of such an effect in these experiments supports the hypothesis that the bacteria are suppressing nematode multiplication by some other mechanism. At least in the case of suppression by *P. aureofaciens* BG33, production of an egg-kill factor is thought to play an important role (38). Nematode suppression at low levels of the biocontrol agent may extend the period of protection between bacterial applications and enhance the potential for rhizobacteria to control nematodes in perennial crops. However, successful long-term survival of an introduced rhizosphere inhabitant on perennials may be difficult to achieve, as "the longer the life of the host, the more perfectly must antagonists be adapted to the rhizosphere" (4).

The low efficiency (2.4%) of recovery of bacteria antagonistic to ring nematode could be improved by identification of characteristics associated with the observed antagonism and permit development of more efficient in vitro bioassays to search for more effective nematode antagonists. To accomplish this, we are currently examining the mode of action of several rhizobacteria in suppressing nematode multiplication.

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