Biological Suppression and Natural Population Decline of *Heterodera schachtii* in a California Field

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ABSTRACT

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Soil suppressiveness to *Heterodera schachtii* was demonstrated in a field at the research station of the University of California, Riverside. In two field trials planted to Swiss chard (*Beta vulgaris*), introduced *H. schachtii* multiplied 2.7 and 1.7 times more in preplant metam sodium-fumigated plots than in nontreated plots in 1994 and 1995, respectively. In greenhouse experiments, preplant treatments with metam sodium, methyl bromide, methyl iodide, formaldehyde, and aerated steam reduced suppressiveness of soil against *H. schachtii* to undetectable levels. *H. schachtii* multiplied significantly less in nontreated soil than in treated soil on

Swiss chard. At harvest, the number of infective second-stage juveniles in suppressive soil was close to the lowest detection level, whereas high numbers were encountered in soils initially treated. In a crop rotation trial with host crops of *H. schachtii*, introduced *H. schachtii* populations were monitored for five cropping periods over 30 months in initially fumigated versus nontreated suppressive field plots. In fumigated plots, *H. schachtii* population levels increased in the first and second cropping periods and then declined in the third cropping period. In the fourth and fifth cropping periods, the nematode reproduction factor in the initially fumigated plots was not significantly different from that in suppressive plots.

Additional keywords: biological control, natural decline, nematode population decline, population dynamics, sugar beet cyst nematode.

Although soil suppressiveness is an often-cited phenomenon (12), relatively little is known about the interactions in soils suppressive to plant-parasitic nematodes. Nematode suppression was most intensively studied with cyst nematodes (6,15). The population decline of the cereal cyst nematode, *Heterodera avenae*, was first reported under intensive cropping of cereal hosts (9). A major role for parasites of eggs and females of *H. avenae* in the nematode population decline was postulated by Kerry and Crump (16). Kerry et al. (17) demonstrated a reduction of nematode suppression after drenching the soil with formaldehyde. Consequently, the authors identified the fungal parasites of female nematodes, *Nematophthora gynophila* and *Verticillium chlamydosporium*, to be important in cyst nematode suppression (18,19). The population decline of *H. schachtii* under continuous monoculture of sugar beets over an 18-year period was described in the Netherlands (11). Fungal parasites were thought to be responsible for the decline. The broad-spectrum fungicide captan reduced the suppression of *H. schachtii* under sugar beets in tests reported by Crump and Kerry (7). In California, *Acremonium strictum* and *Fusarium oxysporum* were isolated from parasitized *H. schachtii* eggs and shown in vitro to destroy eggs of *H. schachtii* (23,24). An antagonistic interaction between *H. schachtii* and a putatively plant-pathogenic *F. oxysporum* mutant was reported under experimental conditions in an independent study (13). However, little is known about the role of *F. oxysporum* and *A. strictum* in the field population dynamics of the sugar beet cyst nematode. Other investigations focused on the suppression of populations of root-knot nematodes, *Meloidogyne* spp. Fungal parasites of eggs and females or the prokaryote *Pasteuria penetrans* were primary determinants of the nematode population decline of *Meloidogyne* spp. (5,25). Other bacteria were found responsible for suppression of root-knot nematodes (29). Rhizo-

bacteria were reported to be involved in soil suppressiveness against *Crictonemella xenoplax* (20).

This project focused on one field at the agricultural research station of the University of California, Riverside. In 1975, a 1-ha field site was amended with *H. schachtii*-infested field soil from the nearby Moreno Valley field station of the University of California. Under continuous cropping of various host plants of the nematode, an initial cyst nematode population of up to 40 eggs per g of soil was established during the first years following infestation (I. J. Thomason, *personal communication*). The cyst nematode population declined over the next several years and has remained at a low level throughout the field. The objective of this study was to systematically describe this phenomenon and determine the nature of the suppressiveness against *H. schachtii* in this field. Preliminary reports of this study have been published (27,28).

MATERIALS AND METHODS

Field confirmation of biological suppressiveness. During 1994 and 1995, two independent field trials were conducted in a soil suppressive against *H. schachtii* at the Agricultural Operations Field Station, University of California, Riverside. The soil was a Hanford fine sandy loam (60.9% sand, 29.6% silt, and 9.5% clay, pH 8) with an initial sugar beet cyst nematode population of 6.2 and 5.6 eggs per g of soil at the beginning of the respective trials. No other plant-parasitic nematodes were detected in significant numbers, and these were not monitored further during this study. After soil preparation with a rototiller and a disk, the 1994 test site was fertilized with 335-kg/ha ammonium phosphate fertilizer (16% N, 20% P, and 13% S). In 1995, the second test site received 335-kg/ha ammonium nitrate fertilizer (34% N). The preplant herbicide pebulate (5.03 kg a.i./ha) was incorporated with a cultmulcher.

In each trial, treatments were arranged in a randomized complete block design with five replications. The individual plots were 5.10 m long and 2.25 m wide, each consisting of three seedbeds on 75-cm centers. The center row was used for monitoring nema-

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tode population densities and plant growth. The following three treatments were established: (i) nontreated, noninfested; (ii) nontreated, infested; and (iii) metam sodium-treated, infested. Metam sodium (356 kg a.i./ha) was diluted in 91 liters of water for each plot and applied with sprinkling cans. The application was done 10 weeks before greenhouse-reared *H. schachtii* were introduced into the treated and nontreated plots. Nematodes were raised in sandy soil on canola, *Brassica napus*. Within each infested plot, 10 soil cores each 10 cm deep were taken with a bucket auger (7.6-cm diameter) in the center of the seedbeds at 30-cm spacing. Each soil core was placed into a 2-liter plastic bag and thoroughly mixed with the greenhouse-reared nematode inoculum containing sugar beet cyst nematodes (approximately 157,000 eggs in 1994; approximately 80,000 eggs in 1995) in sandy soil (275 and 250 g, respectively). The soil mix was then replaced into its original hole. The nontreated, noninfested control received only the sandy soil in which canola was grown without the nematodes.

The plots were irrigated with overhead sprinkler irrigation to maintain a water potential of -20 to -30 kPa. One month after the nematode infestation, Swiss chard, *Beta vulgaris* ('Paros'; Shepherd's Garden & Seeds, Felton, CA), was seeded into the center of each infested planting site or appropriate site in noninfested control plots. Shortly after emergence, the plants were thinned to one plant per planting site. During the season, insects were controlled with carbaryl (1.12 kg a.i./ha) in 1994 and imidacloprid (52.5 g a.i./ha) in 1995. The crop was grown for approximately 950 degree-days (DD, to the basal temperature of 8°C at a depth of 10 cm) in 1994 and 1,035 DD in 1995. This was sufficient for the development of two nematode generations (8). At harvest, the plant tops were cut off at the soil line and oven dry weights were determined. The roots were recovered along with the adhering soil core with a bucket auger (7.6-cm diameter) to a depth of 10 cm. Adhering cysts and soil were washed from the roots over a U.S. standard (USS) sieve no. 20 (0.83 mm) nested onto a USS sieve no. 100 (0.15 mm). Roots were oven-dried and weighed. The recovered wash-off material was air-dried and remixed into the remainder of the soil core from which it originated. A 350-g subsample was processed in a modified Fenwick flotation can (4) with an extraction efficiency from the moist soil of approximately 80%. Cysts were counted and broken in a tissue homogenizer to release the eggs for enumeration. The arithmetic means of the subsamples of each plot were used for statistical analysis.

Monitoring soil treatments and cyst parasites. At the time of the planting site infestation, 60 samples were taken from each plot with an Oakfield soil tube (Oakfield Apparatus Company, Oakfield, WI) to a depth of 10 cm, pooled, and thoroughly mixed. A 50-g subsample from each plot was air-dried. After screening through a USS sieve no. 40 (0.425 mm), the dried samples were used for dilution plating on two different agar media. Soil samples were dilution-plated on Komada's selective medium (21) to monitor the treatment effect on *F. oxysporum*. Additionally, the soil samples were dilution-plated on modified Mircetich's selective medium (50 mg of rifampicin, 100 mg of pentachloronitrobenzene, 10 mg of rose bengal, 20 g of sucrose, 1 mg of ZnCl₂, 10 mg of MgSO₄ · 7H₂O, 10 mg of CaCl₂, 17 g of cornmeal agar, 23 g of agar, and 1 liter of water) (22) to monitor treatment effectiveness against *Pythium* spp. Depending on the organism to be examined, between 0.25 to 3.0 g of soil were mixed into 20 to 50 ml of water agar (0.25%). The arithmetic means of the three subsamples per plot were used for statistical analysis.

In the 1995 trial, 100-g subsamples of the soil collected at harvest were used to recover cysts for an examination of the fungal parasitism of the cysts. Each of the soil samples was placed into a 1,750-ml cup and swirled up with a jet stream of water. The supernatant was then decanted into a USS sieve no. 100 (0.15 mm) screen nested onto a 7-liter bucket, and the procedure was repeated until the bucket was filled. Cysts were hand-picked at random from the screen residue under a dissecting microscope. For each soil

sample, 100 cysts were squashed between glass plates and examined for fungal infestation under a compound microscope. Cysts were divided in terms of parasitism: *a* = nonparasitized, no significant hyphae visible in cyst contents; *b* = moderately parasitized, approximately half the cyst contents infested with fungal hyphae; and *c* = highly parasitized, entire cyst contents filled with fungal hyphae. The data were calculated as frequencies of the classes per treatment and then expressed as percent infestation (*P*) in the cyst population examined with the formula $P = 0.5 \cdot b + c$. The percent infestation (*P*) was used to calculate the analysis of variance (ANOVA).

Bioassay for nematode infectivity. Three subsamples (90 g dry weight each) of the composite soil sample from each plot from the 1995 harvest were each placed in a 250-ml polystyrene container. Each of the subsamples was adjusted to 11% moisture content, and the containers were closed with a polyethylene lid to reduce evaporation and left overnight to allow moisture adjustment throughout the soil. Radish seeds, *Raphanus sativus* ('Cherry Belle'; Ferry-Morse Seed Co., Fulton, KY), were surface-sterilized (5 min in 20% commercial bleach, 5.25% NaOCl) and single-seeded into each container. The containers were covered again and incubated for 4 days at 28 to 23°C with a 16/8-h day/night cycle in an environmental growth chamber. The roots of the radishes were cleared and stained for juvenile detection (2). The means of the three subsamples per original field plot of second-stage juveniles (J2) per cm of root length were used for statistical analysis.

Greenhouse experiments. Soil from the suppressive field was randomly collected to a depth of 10 cm with a shovel, passed through a 0.32-cm-pore sieve, and mixed 10:1 (dry weight) with silica sand in a cement mixer. Twenty-kilogram portions of this soil were placed in 19-liter, high-density polyethylene buckets. Soil moisture at treatment was gravimetrically determined to be 7%. Each bucket represented one of six treatments: nontreated control; metam sodium (356 kg a.i./ha); methyl bromide (335 kg a.i./ha); methyl iodide (503 kg a.i./ha); formaldehyde solution, 37% with 10 to 15% methanol (3,000 liters/ha); and heat treatment with aerated steam at 87.5°C for 30 min (1). Rates for the chemical treatments were calculated per surface area of the soil column. Metam sodium and formaldehyde were applied as a drench in 2 liters of water. The fumigants (methyl bromide cooled to -56°C) were pipetted into small vials and placed on top of the soil. The buckets were immediately covered with black, 0.1-mm-thick polyethylene tarp that was secured by a large rubber band for 5 days.

Ten days after the chemical treatments and one day after treatment with aerated steam, equivalents of 2.9 kg dry weight of soil were placed into 15-cm pulp pots (Western Pulp Products Co., Corvallis, OR) and soil moisture was adjusted to 11%. The pots were incubated in the greenhouse (natural day/night cycle at 24 ± 3°C) in a randomized complete block design with five replications. The trial was irrigated with a low volume irrigation system (Netafim Irrigation, Inc., Fresno, CA) with battery-powered timer controls (Gardena; Aquapore Moisture Systems, Inc., Phoenix, AZ). One 5-week-old seedling of Swiss chard ('Large White Ribbed'; Lockhart Seeds, Inc. Stockton, CA) was planted into each pot. Each plant was fertilized with 50 ml of nutrient solution (10 g of Miracle-Gro per 3.79 liters of water, 15% N, 30% P, 15% K; Scotts Miracle-Gro Products, Inc., Port Washington, NY) weekly. Two weeks after planting, the pots were infested with 5,000 *H. schachtii* J2.

Eleven weeks after infestation, after approximately two nematode generations, the experiment was harvested. Plant tops were cut off at the soil line and oven-dried. Nematode cysts and soil were washed off the roots and mixed with the remaining soil of the pot. Cysts were extracted from 350-g subsamples of soil from each pot, and cysts and eggs were counted. Oven dry weights of the tops and roots were determined. Three subsamples (54 g dry weight each) from each pot were placed into 250-ml cups, adjusted to 11% moisture, and used for a radish bioassay as described above but with a 3-day incubation time. The number of *H. schachtii* J2

detected in the bioassay was expressed as percentage of invasion (I) of the nematode eggs present in the soil at harvest, based on the formula $I = (100 \cdot J2 \text{ per cm}) / (60 \cdot \text{eggs per g of soil})$. The percentage of invasion (I) was used for ANOVA. This greenhouse experiment was conducted twice.

Continuous host-cropping field trials. The nematode populations that were introduced into nontreated, suppressive plots and fumigated plots were monitored for five cropping periods in the 1995 trial site. The fumigated plots will be called conducive plots hereafter. After each crop, the seedbeds were rototilled to a depth of 10 cm and reshaped for the following crop. The crop rotation sequence was Swiss chard in summer 1995; canola, *B. napus*, in winter 1996; Swiss chard in summer 1996; cabbage, *B. oleracea*, in winter 1997; and Swiss chard in summer 1997 (Table 1). Crops were grown to permit about two nematode generations to develop (850 to 878 DD in winter; 1,195 to 1,560 DD in summer). The first Swiss chard and the canola crops were direct-seeded, and the later Swiss chard and cabbage crops were established as 5-week-old transplants.

For each cropping period, pre-season samples were taken with an Oakfield soil tube to a depth of 10 cm (20 subsamples total). At canola harvest, a set of 40 subsamples (20 per plant row) was taken from the root zone of each plot and pooled. At the following three harvests, four subsamples of soil were taken from each planting site with an Oakfield sampling tube 2.5 cm from the plant stem to a depth of 10 cm. These subsamples were combined to one sample per plot. After thoroughly mixing the soil in plastic bags, cysts were extracted from two subsamples per composite sample. Nematode cysts and eggs were counted. The arithmetic means of the two subsamples were used for statistical analysis. Plant top and root dry weights were determined after oven drying, except for the top weights of canola and cabbage from which fresh weights were obtained.

A second trial, adjacent to trial 1, with a split block design with six replications was started in spring 1996. The seedbed spacing was the same as in field trial 1. The second trial had nontreated, suppressive plots and conducive plots that were hot gas-fumigated with methyl bromide (10). For hot gas-fumigation drip-irrigation tubing (1.25-cm diameter, 30-cm emitter spacing, and 2-liter/h emitter capacity; Drip In Irrigation Co., Fresno, CA) was placed on the surface in the middle of each seedbed. The beds were covered with clear, 0.025-mm-thick polyethylene tarp. The edges of the tarp were buried with soil in the trenches between the beds. Methyl bromide

(336 kg a.i./ha) was heated in a contained system and applied through the drip lines with nitrogen as the carrier gas. The tarps were removed after 5 days and the plots kept moist with the drip irrigation. Six weeks later, greenhouse-reared cyst nematodes (50,000 eggs per planting site) were incorporated into 10 planting sites per plot as described above. Immediately after infestation, the plots were irrigated to keep the soil water potential at -20 to -30 kPa. Crops planted were Swiss chard in summer 1996, cabbage in winter 1997, and Swiss chard in summer 1997. All crops were established as transplants (Table 1). The crops were grown for a sum of DD required for about two sugar beet cyst nematode generations to develop (878 DD in winter; 1,039 and 2,000 DD in summer). After each crop, the seedbeds were rototilled to a depth of 10 cm and reshaped for the following crop. At the first harvest, the tops were cut off at ground level and the soil cores around the host roots, the planting sites, were recovered with the bucket auger as described above. In the second and third cropping periods, the sampling was done as described for the later samplings of trial 1. Sample processing, nematode enumeration, and plant harvest data collection were done as described above. For both trials, the nematode reproductive factor (R) was determined by dividing the number of *H. schachtii* eggs at harvest (Pf) by the number of *H. schachtii* eggs at planting (Pi) of the respective plots ($R = Pf/Pi$).

Greenhouse assessment of nematode suppressiveness in populations of *H. schachtii*. At the last planting of both field trials (May 1997), trials 1 and 2 had been cropped for four or two cropping periods, respectively, with host crops of *H. schachtii*. One soil sample from each of the suppressive and initially conducive plots of trials 1 and 2 was collected with a trowel from the upper 10-cm soil layer. Based on the individual population levels, the soil samples from each plot were diluted with methyl iodide-fumigated soil from the same field to obtain a uniform nematode population of 12.9 eggs per g of soil. This was done irrespectively of the nematode cyst numbers per g of soil. Silica sand was added to each sample (10:1, wt/wt). For each field plot, two 15-cm pots were prepared with an equivalent of 2.9 kg of soil dry weight and then adjusted to a moisture content of 11%. The pots were planted with 5-week-old seedlings of Swiss chard 'Large White Ribbed'. This greenhouse experiment was arranged in a randomized complete block design in ambient light at $24 \pm 3^\circ\text{C}$. After 4 weeks, one pot of each soil was infested with 7,500 *H. schachtii* J2 suspended in

TABLE 1. General agricultural practices in continuous host-cropping trials 1 and 2

Trial/ period	Cropping period	Crop ^p	Planting ^q			Fertilizer ^r		Herbicide ^s		Insecticide ^t	
			Rows	Spacing	Method	NH ₄ NO ₃ ^u	N-P-K	Material	kg a.i./ha	Material	g a.i./ha
Trial 1											
1st	8/95 to 10/95	Swiss chard	1	30	S	335		Pebulate ^v	5.0	Imidachloprid	52.5
2nd	12/95 to 4/96	Canola	2	15	S	176	52 ^w			Acephate ^x	839.5
3rd	6/96 to 8/96	Swiss chard	1	30	T	88	52 ^w	Diquat dibromide ^v	0.6	Imidachloprid	2 · 52.5
4th	10/96 to 3/97	Cabbage	1	30	T		333 ^y	Diquat dibromide ^v	0.6	Imidachloprid	52.5
							156 ^w	Glyphosate ^z	1.4	Acephate	1,119.4
5th	5/97 to 8/97	Swiss chard	1	30	T		400 ^y	Glyphosate ^v	1.4	Imidachloprid	52.5
							104 ^w				
Trial 2											
1st	6/96 to 8/96	Swiss chard	1	30	T		336 ^y	Imidachloprid	52.5		
2nd	10/96 to 3/97	Cabbage	1	30	T		333 ^y	Diquat dibromide ^t	0.6	Imidachloprid	52.5
							156 ^w	Glyphosate ^z	1.4	Acephate	1,119.4
3rd	5/97 to 8/97	Swiss chard	1	30	T		400 ^y	Glyphosate ^t	1.4	Imidachloprid	2 · 52.5
							208 ^w				

^b Swiss chard, *Beta vulgaris*; canola, *Brassica napus*; and cabbage, *Brassica oleracea*.

^a Rows = rows per seedbed; Spacing = plant distance within plant row (cm); S = seeding; and T = transplant.

^c Fertilizer were applied as granular fertilizer or liquid fertilizer, amounts in kg/ha per season are shown.

^d Herbicide use was complemented with mechanical weed control.

^e Insecticides were applied as broadcast spray application, unless otherwise indicated.

^u Granular fertilizer, ammonium nitrate 34% N.

^v Preplant treatment with herbicide.

^w Miracle-Gro 15% N-30% P-15% K; Scotts Miracle-Gro Products, Inc., Port Washington, NY.

^x Spot treatment with the insecticide indicated.

^y Triple Pro 15-15-15 + 10% sulfur; J. P. Simplot Co., Lathrop, CA.

^z Spray application during cropping period in furrows.

20 ml of water (set A); the second pot received only water (set B). Eleven weeks after infestation, the experiment was harvested and the top and root dry weights were determined and nematode data were collected as previously described for greenhouse experiments.

Data analysis. All data from all experiments were subjected to ANOVA with SuperANOVA (Abacus Concepts, Inc., Berkeley, CA). Fisher's protected least significant difference (LSD) test was used to separate means at $P = 0.05$ if treatment F had $P \leq 0.05$. The test was performed at $P = 0.10$ if treatment F had $0.05 \leq P \leq 0.10$. Data from the repetition of the greenhouse experiment on the evidence of suppressiveness were combined for statistical analysis because of the similarity of results in the two tests. The two trials on the effect of continuous host cropping were initially analyzed as original data. The average of the values of the suppressive plots for each year was used as a 100% standard. All values were computed with the formula $x = 100 \cdot y/z$, in which x = standardized value, y = original measurement, and z = mean of measurements in suppressive plots. ANOVA was repeated and the means were compared with Fisher's protected LSD test. The two sets of the greenhouse assessment of suppressiveness were calculated separately for the nematode inoculated (A) and noninoculated (B) sets.

RESULTS

Field confirmation of biological suppressiveness. In the field trials, both the number of cysts per g of soil and the number of eggs per g of soil were significantly higher in the infested, fumigated soil compared with those in the infested, nontreated soil in both years. More cysts and eggs per g of soil were detected in the infested controls than in the noninfested controls (Table 2). In 1994, the number of eggs per cyst was significantly higher in the fumigated treatment than in the controls. The difference in the number of eggs per cyst in the trial in 1995 was much smaller and not significant (Table 2). However, invasion of radish roots by J2 nearly doubled in fumigated soil ($P = 0.10$) (Table 2). Fungal infestation of the cysts was significantly higher in the nontreated controls compared with a low infestation in the fumigated soil (Table 2). In both years, differences between the top and root dry weights of the infested treatments were not significant (Table 2, only root dry weights shown). In both years, the *F. oxysporum* and *Pythium* spp. populations were significantly reduced by the metam sodium treatment (Table 3).

Greenhouse experiments. The number of cysts per g of soil was significantly higher in all soil treatments with biocides than that in the nontreated, suppressive control, although plants in all pots had received the same nematode inoculum. The average number of eggs per g of soil that had received biocide treatments was 9.5-fold higher than that in the control (Table 4). After each biocidal treatment, significantly more J2 were detected in the bioassay roots of radish than in the control. The percentage of root invasion (I) averaged 8.8-fold higher than that in the nontreated, suppressive control.

Top and root dry weights were higher in all the biocidal treatments compared with those in the nontreated control.

Continuous host-cropping field trials. Nematode egg numbers were higher in the initially conducive plots than in the suppressive plots in the first cropping period of both trials and much higher in the second cropping period. In the third and subsequent cropping periods, the nematode populations declined in the initially conducive plots in both trials (Fig. 1A and B). The numbers of eggs per cyst were significantly higher in the conducive soil than in the suppressive soil in the first and second cropping periods in both trials and in the third cropping period in trial 2 (Fig. 1C and D). In trial 1, the number of eggs per cyst declined in the third cropping period below that in the suppressive soil and then remained at the level of that in the suppressive plots (Fig. 1C).

The reproductive factor (R) of the nematode eggs in the conducive soil was significantly higher than that in the suppressive soil in the first and second cropping periods in both trials. In the third cropping period, the R value dropped to the level of that in the suppressive plots (Fig. 1E and F). In trial 2, the R value dropped below that of the suppressive plots in the third cropping period ($P \leq 0.10$) (Fig. 1F). The number of cysts per g of soil was significantly higher in the conducive plots than in the suppressive plots during the entire length of both trials (Fig. 1G and H). The greatest difference in cyst numbers was observed in the third cropping period (2.1-fold) of trial 1.

In the first trial, the plant top weights were not significantly different among the treatments in the first cropping period. They were significantly lower in the conducive soil than in the suppressive plots in the second ($P \leq 0.10$) and third ($P \leq 0.05$) cropping periods and were significantly higher in the initially conducive soil than in the suppressive soil in the fifth cropping period ($P \leq 0.05$). In trial 2, there were no significant differences among the treatments in top weights in the first and second cropping periods. In the third cropping period,

TABLE 3. Colony forming units (CFU) of *Fusarium oxysporum* and *Pythium* spp. after preplant treatments in 1994 and 1995 field trials^y

Preplant treatment ^z		CFU <i>F. oxysporum</i> × 10 ⁴ per g of soil		CFU <i>Pythium</i> spp. per g of soil	
Fumigation	Infestation	1994	1995	1994	1995
–	–	1.7 b	0.6 b	54.2 b	21.8 b
–	+	1.6 b	0.5 b	43.6 b	26.7 b
+	+	0.3 a	0.2 a	0.9 a	0.0 a
LSD, $P = 0.05$		0.9	0.3	21.8	17.4
P for treatment F		0.0167	0.0070	0.0011	0.0001

^y For each column, treatments followed by the same letter were not significantly different according to Fisher's protected least significant difference (LSD) test at $P = 0.05$.

^z Fumigation = preseason metam sodium fumigation (356 kg a.i./ha); Infestation = introduction of *Heterodera schachtii*.

TABLE 2. Effect of fumigation of a *Heterodera schachtii*-suppressive soil on root weight, *H. schachtii* population densities, second-stage juveniles (J2) present in radish roots, and fungal infestation of cysts^x

Preplant treatment ^y		Root dry weight (g)		Nematode cysts/g of soil		Eggs/g of soil		Bioassay (J2/cm of root) ^z	Fungal cyst infestation (%)
Fumigation	Infestation	1994	1995	1994	1995	1994	1995	1995	1995
–	–	6.5 b	7.2 b	0.6 a	0.7 a	8.9 a	19.3 a	11.0 a	82.6 b
–	+	5.5 ab	3.9 a	1.1 b	1.1 b	32.3 b	37.9 b	37.3 ab	88.8 b
+	+	3.7 a	4.0 a	2.0 c	1.5 c	87.8 c	59.5 c	68.1 b	32.6 a
LSD, $P = 0.05$									
LSD, $P = 0.10$		1.8		0.3	0.2	11.1	9.4	36.4	12.7
P for treatment F		0.0552	0.0353	0.0001	0.0001	0.0001	0.0001	0.0203	0.0001

^x For each column, treatments means followed by the same letter were not significantly different according to Fisher's protected least significant difference (LSD) test at the level of probability indicated.

^y Fumigation = preseason metam sodium fumigation (356 kg a.i./ha); Infestation = introduction of *H. schachtii* (approximately 157,000 eggs in 1994; approximately 80,000 eggs in 1995) within sandy potting mix.

^z *H. schachtii* J2 detected in radish roots after a 4-day incubation of a 16/8-h (day/night) cycle at 28/23°C in different soils. Mean separation at $P = 0.05$ indicated by letters.

the plant top weights in the initially conducive soil were increased compared with those in the suppressive soil ($P \leq 0.05$) (data not shown). During trial 1, root dry weights were not significantly different. In trial 2, the root weights in the initially conducive plots were significantly ($P \leq 0.05$) higher than those in the suppressive plots in the first and third cropping periods (data not shown).

Greenhouse assessment of nematode suppressiveness in populations of *H. schachtii*. For soils from trials 1 and 2 infested during greenhouse incubation (set A), numbers of cysts per g of soil were not significantly different in the conducive and suppressive soils obtained from field trial 1 or 2. However, cyst numbers in the suppressive soil from field trial 2 were significantly lower than those in both soils from trial 1. The numbers of cysts per g of soil were the same in the initially conducive soils collected from both trials (Table 5). The suppressive soil from trial 1 had the highest egg population and the initially conducive soil of trial 2 had the lowest (Table 5). The number of eggs per cyst in the conducive soil of trial 2 was lower than that in the other soils. Only in the conducive soil from trial 2 was the reproductive factor ($R = \text{Pf/Pi}$) of the cysts significantly higher than in the other soils. In general, plant weights were not significantly different among the different treatments. Only the plants grown in the suppressive soil from trial 2 had reduced top weights in comparison to those in the other soils (Table 5). There were no significant differences in root weights, and none were detected in either the numbers of cysts or eggs per g of soil, the numbers of eggs per cyst, or plant weight data in set B, which had not been infested additionally during greenhouse incubation.

DISCUSSION

Removal of biological suppressiveness in greenhouse and field trials as well as the decline of *H. schachtii* population densities

under continuous host cropping demonstrated the biological nature of suppressiveness against *H. schachtii* in the examined California field. The suppressiveness was eliminated by soil fumigation of field plots in 2 consecutive years. In greenhouse experiments, different fumigants and other biocidal treatments again resulted in eliminating the suppressiveness. Removal of suppressiveness by biocidal treatments was previously used to demonstrate the biological nature of nematode-suppressive soils (7,11,17–19,29). Most often, fungi have been implicated in cyst nematode suppression. Some potential nematode-destroying fungi have been tested for their sensitivities towards fungicidal and biocidal treatments (14).

In our research, higher numbers of eggs per cyst following the biocidal treatment of the soil were observed throughout the trials. This suggested an impact of suppressiveness against the females, cysts, and eggs of the nematode as demonstrated for cereal cyst and sugar beet cyst nematode-suppressive soils in Europe (7,11, 16,18,19). The parasitism of eggs of *H. schachtii* by *F. oxysporum* has been reported in California (23,24), and an antagonistic interaction between *F. oxysporum* and *H. schachtii* has been reported (13). Our monitoring data of *F. oxysporum* indicated that potentially it could play a role in the *H. schachtii* suppressiveness.

In our trials, twice as many infective juveniles were detected in the root zones of the assay plants grown in soil from pre-season fumigated, infested plots than in those grown in nontreated, infested field plots. In the greenhouse experiments, removal of suppressiveness by biocidal treatments increased the number of infective nematode juveniles more than 100-fold in comparison to that in the control. The number of infective juveniles, therefore, increased more than proportionally to the number of eggs per g of soil. This suggested an impact of suppressiveness on the infectivity of the nematode juveniles. The high fungal infestation of the field-derived cysts from the suppressive plots supported the hypothesis of an

TABLE 4. Effect of biocidal treatments of nematode-suppressive soil on plant growth, *Heterodera schachtii* population density, and frequency of second-stage juvenile (J2) invasion into roots in greenhouse experiments¹

Preplant treatment	Greenhouse experiments					Bioassays	
	Top dry weight (g)	Root dry weight (g)	Cysts/g of soil	Eggs/g of soil	Eggs/cyst	J2/cm of root length	J2 invaded (%) ²
Control	3.8 a	0.4 a	0.5 a	21.2 a	34.2 a	0.6 a	0.1 a
Metam sodium	7.5 b	4.6 c	1.3 bc	220.3 cd	166.8 c	59.1 b	0.7 b
Methyl bromide	8.8 c	5.2 cd	1.3 bc	202.3 bcd	151.1 bc	94.7 bc	0.9 b
Methyl iodide	9.1 c	6.0 d	1.3 bc	181.7 bc	136.2 b	96.1 bc	1.0 b
Formaldehyde	6.9 b	3.5 b	1.5 c	255.1 d	152.2 bc	57.3 b	0.8 b
Aerated steam	9.7 c	5.5 cd	1.1 b	150.7 b	131.8 b	118.9 c	1.5 c
LSD, $P = 0.05$	1.2	0.3	0.3	61.2	29.8	41.8	0.4
P for treatment F	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

¹ For each column, treatment means followed by the same letter were not significantly different according to Fisher's least significant difference (LSD) test at $P = 0.05$. Data from two tests were combined for statistical analysis.

² Percentage of *H. schachtii* J2 detected per cm of root length of radish in relation to the number of *H. schachtii* eggs present in the 60-g soil sample.

TABLE 5. Greenhouse assessment of suppressiveness of soil from field trials 1 and 2 after continuous host cropping, collected at the beginning of the fifth (trial 1) or third (trial 2) cropping period^w

Trial ^x	Cysts/g of soil	Eggs/g of soil	Eggs/cyst	Cysts: Pf/Pi ^y	Top weight (g)	Root weight (g)
Trial 1 soil						
Suppressive	1.3 c	52.1 b	38.9 b	2.9 a	11.7 b	1.8 a
Conductive	1.1 bc	36.2 ab	33.6 ab	2.2 a	12.3 b	1.8 a
Trial 2 soil						
Suppressive	0.7 a	29.3 a	37.7 b	2.7 a	7.3 a	1.2 a
Conductive	0.9 ab	23.5 a	25.4 a	9.5 b	11.7 b	1.3 a
LSD, $P = 0.05$	0.3	15.5		2.5	2.8	NS ^z
LSD, $P = 0.10$			8.9			
P for treatment F	0.0133	0.0142	0.0558	0.0010	0.0277	0.5226

^w All soils were adjusted to an egg content of 12.9 eggs per g of soil by diluting with methyl iodide-fumigated field soil. Three weeks after planting, the pots were infested with 7,500 second-stage juveniles of *Heterodera steatite*. For each column, treatment means followed by the same letter were not significantly different according to Fisher's protected least significant difference (LSD) test at the indicated probability levels.

^x Suppressive and Conductive refer to the status of the soil at the initiation of the field trial.

^y Reproductive factor of cysts = number cysts at harvest (Pf) divided by number of cysts at planting of greenhouse experiment (Pi).

^z Not significantly different.

important role of fungal female, cyst, and egg parasites in this suppressive soil as described for other cyst nematode-suppressive soils (3,16).

The reduced plant weights in the suppressive controls of the greenhouse experiments were probably due to root pathogens. The poor root growth was a constant problem when working with field soils in greenhouse pot experiments and may have contributed to the diminished nematode multiplication. Crump and Kerry (7) reported the reduction of nematode multiplication due to the loss of feeder roots in suppressive soils.

The continuous host cropping of the initially conducive plots resulted in a classical decline of nematode populations in the third cropping period as described for other nematode pest population declines (9,11,14). In our trials, the decline of *H. schachtii* populations was observed after 18 months. The development of suppressiveness could have been favored by the use of a suppressive field site for the tests, where a potential horizontal recolonization of the initially fumigated plots could not be completely excluded. In addition to the difference in the biology of cyst nematode species, climatic conditions in southern California permit frequent generations

of *H. schachtii* (26), which might have decreased the time necessary for the decline process to occur. In this population decline, the reproductive factor dropped below the value of the suppressive plots, indicating that the plots regained suppressiveness. Differences between the nematode population developing in initially suppressive and those in initially conducive soils were not significantly different when these soils were adjusted to the same nematode population in the greenhouse experiments testing the status of suppressiveness. These observations supported the conclusion that the soils regained suppressiveness.

The highest cyst multiplication occurred in the initially conducive soil of trial 2. Newly developed cysts were no longer filled with high numbers of eggs as found in truly conducive soils in the biocidal treatment greenhouse experiments. This indicated that the conditions for nematode reproduction became less favorable during this growing period, especially in the second generation of the nematode. Similar observations have been reported from studies on *H. schachtii* in sugar beet monoculture (7). The lower number of cysts per g of soil in the suppressive soil of field trial 2 compared with that in field trial 1 could indicate that suppressiveness

Fig. 1. Development of *Heterodera schachtii* in suppressive and initially conducive plots. The means of the suppressive plots were set as a 100% standard before analysis of variance, followed by Fisher's protected least significant difference separation of means. Significant differences within each cropping period were indicated with * ($P = 0.05$) and † ($P = 0.10$). Bars represent standard errors. Nematode reproductive factor ($R = Pf/Pi$) was the number of eggs at harvest (Pf) divided by the number of eggs at planting (Pi).

was enhanced in the soil with the less recent disturbance of the steady state of cyst nematodes and their antagonists. Further studies to confirm the involvement of female, cyst, and egg parasites in this suppressiveness are ongoing.

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