SOIL SUPPRESSIVENESS AGAINST *HETERODERA SCHACHTII* IN CALIFORNIA CROPPING AREAS

A. Westphal*[1], A. Pyrowolakis[2], R. A. Sikora[2], and J. O. Becker[3]

[1]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, 48161 Münster, Germany; [2]INRES-Phytomedicine, Soil-Ecosystem Phytopathology & Nematology, University of Bonn, 53115 Bonn, Germany; [3]Department of Nematology, University of California, Riverside, CA 92521, USA. Corresponding author: andreas.westphal@jki.bund.de

ABSTRACT

Westphal, A., A. Pyrowolakis, R. A. Sikora, and J. O. Becker. 2011. Soil suppressiveness against *Heterodera schachtii* in California cropping areas. Nematropica 41:161-171.

A greenhouse bioassay was developed for determining the presence of biological suppressiveness against the beet cyst nematode, *Heterodera schachtii*, using eleven soils from southern California locations. Two soils exhibited *H. schachtii*-suppression of nematode population development in non-treated compared to methyl iodide-fumigated (500 kg/ha) equivalents. By counting nematode cysts and eggs, suppressiveness was detectable on Swiss chard after two generations of *H. schachtii* following infestation with second-stage juveniles (J2) of *H. schachtii* or on mustard-greens after infestation with nematode cysts. In another test, enumeration of nematode females visible through the transparent pot wall was a useful method for observing *H. schachtii* populations. In a survey with mustard-greens, two of 48 soils from southern California were suppressive against *H. schachtii*, indicated by reduced numbers of eggs or cysts of *H. schachtii* in non-treated portions compared to fumigated equivalents. The data suggested that various soil textures allow for suppressiveness against *H. schachtii* because of the four suppressive soils (of 59 total) two were loam soils, one a clay loam, and one a sandy loam. The number of nematode females visible through the transparent pot surface was correlated with the number of extractable cysts 100 g^{-1} soil ($R^2 = 0.45$; $P < 0.01$). Thus, female counts provided estimates of the final *H. schachtii* population density. There was no relationship between the rates of fungal infection of the eggs and the numbers of eggs/cyst at harvest in the greenhouse tests, suggesting that such data are unsuitable predictors of soil suppressiveness for this nematode.

Key words: biological control, nematode egg-parasitic fungi, *Heterodera schachtii*, soil suppressiveness, sugar beet, sugar beet cyst nematode

RESUMEN

Westphal, A., A. Pyrowolakis, R. A. Sikora, and J. O. Becker. 2011. Supresividad del suelo contra *Heterodera schachtii* en áreas agrícolas de California. Nematropica 41:161-171.

Se desarrolló un bioensayo en invernadero para determinar la presencia de supresividad biológica contra el nematodo quiste de la remolacha azucarera, *Heterodera schachtii*, en once suelos agrícolas del sur de California. Se encontró supresividad comparable a tratamientos de fumigación con yoduro de metilo (500 kg/ha) en dos suelos. Se detectó supresividad, medible en cantidad de quistes y huevos, en acelga al cabo de dos generaciones de *H. schachtii* luego de infestación con juveniles de segundo estadio (J2) de *H. schachtii* y en mostaza luego de infestación con quistes del nematodo. En otra prueba, se encontró que el conteo de hembras visibles a través de macetas transparentes fue un método útil para observar poblaciones de *H. schachtii*. En un muestreo de suelos sembrados con mostaza, se encontró supresividad en dos de 48 suelos del sur de California, indicados por número reducido de huevos y quistes de *H. schachtii* en porciones no tratadas comparados con porciones fumigadas. Los resultados sugieren que la supresividad puede hallarse en diferentes texturas de suelo, pues de los cuatro suelos supresivos (de un total de 59) dos fueron suelos limosos, uno arcilloso, y uno limo arenoso. La cantidad de hembras visibles a través de la superficie transparente de la maceta se correlacionó con la cantidad de quistes extraíbles en 100 g^{-1} de suelo ($R^2 = 0.45$; $P < 0.01$). Es decir que el número de hembras brinda un estimativo de la densidad de población final de *H. schachtii*. No se observó relación entre las tasas de infección por hongos y la cantidad de huevos/quistes al momento de cosecha en las pruebas de invernadero, lo cual sugiere que estos datos no son predictores confiables de la supresividad del suelo a este nematodo.

Palabras clave: control biológico, *Heterodera schachtii*, hongos parásitos de huevos de nematodos, nematodo quiste de la remolacha azucarera, remolacha azucarera, supresividad del suelo.

INTRODUCTION

Agricultural soils are believed to have a biological constitution that determines their receptivity to plant diseases and pests (Alabouvette, 1986; Baker and Cook, 1974; Garrett, 1955). With regard to plant-parasitic nematodes, certain groups of soil organisms hypothetically contribute to an antagonistic potential against nematode pests (Stirling, 1991). It is conceivable that population densities of plant-parasitic nematodes can be managed by manipulating the soil environment in favor of such an antagonistic potential (Sikora, 1992). Initially, some positive results in reducing plant-parasitic nematode populations by trapping fungi were reported (Linford *et al*., 1938; Stirling and Smith, 1998), but difficulties in stimulating and supporting their activity remained (Jaffee, 2000). Egg and female parasites were later proposed as biocontrol organisms with higher efficacy potential (Kerry, 1987). One of the best-described interactions between a plant-parasitic nematode and its antagonists is the natural control of the cereal cyst nematode *Heterodera avenae* Woll. in England (Kerry *et al*., 1980). Two fungi, *Nematophthora gynophila* Kerry and Crump and *Pochonia chlamydosporia* Goddard (formerly *Verticillium chlamydosporium*), were primarily responsible for keeping *H. avenae* population densities below economic damage threshold levels (Kerry and Crump, 1980). Nematode suppressive soils were reported for other cyst nematodes and in other areas of the world (Chen 2007a; Crump, 1989; Kerry, 1987; Stirling, 1991; Westphal and Becker, 1999). In contrast, nematode suppression is not consistently effective against *H. schachtii* in the UK, and population densities of the nematode can remain above the damage threshold level in other nematode-suppressive soils (Crump and Kerry, 1987).

Soil suppressiveness against fungal and bacterial diseases is frequently associated with certain soil physical and chemical characteristics (Baker and Cook, 1974). Different microbial communities inhabited soils of different textures (Stotzky and Rem, 1966), which may be an important element for the development of disease suppressive soils. For example, soils with a significant content of montmorillonit-like clay minerals were considered long-term soils for banana because they suppressed the development and spread of the Fusarium wilt of banana (Stotzky and Martin, 1963). Nematode genera may be directly impacted by soil texture composition, e.g., *Meloidogyne* spp., prefer lighter textured soils because of the pore size distribution and other soil physical characteristics (Jones, 1975; Norton, 1979). Indirect effects via microbial activity were surmised as well. For example, sting nematodes, *Belonolaimus longicaudatus* Rau, proliferated in muck soil only after steaming or fumigating the soil with a mixture of 1-3 Dichloropropene and1-2 Dichloropropane (DD) or methyl bromide which suggested a biological cause for the population suppression (Rhoades, 1980). Studies on the ecology of nematophagous fungi showed that non-spontaneous trap-forming predatory fungi were more abundant at lower soil moisture and organic matter content, whereas spontaneous trap-forming predatory fungi were more frequently found in soils with high moisture and organic matter content (Gray, 1985).

Monoculture of susceptible hosts of the primary pathogen is often found to increase the chance for a suppressive soil to develop (Baker and Cook, 1974). Monoculture of hosts facilitated the development of suppressive soils against the cereal, soybean, and sugar beet cyst nematodes (Chen *et al*., 2007a; Kerry, 1987; Westphal and Becker, 1999; Xing and Westphal, 2009; Xing and Westphal, 2006a). Such practice does not always result in an induced soybean cyst nematode-suppressive soil, and long-term monoculture of susceptible soybean may not lead to a decline of *H. glycines* (Chen, 2007b). On the other hand, some soils can be suppressive to soybean cyst nematode, although they are in a rotation of soybean with the non-host corn (Xing and Westphal, 2006b). Currently, it is not possible to predict the specific conditions that may lead to the development of soil suppressiveness against cyst nematodes. One may hypothesize that similar conditions are required as in the classical disease triangle: a susceptible host, an infective pathogen and conducive environmental conditions.

For the past decade, a beet cyst nematode-suppressive soil has been studied in field 9E at the University of California Riverside Agricultural Experiment Station (Westphal and Becker, 1999). It is remarkable that the stability of the *H. schachtii* population suppression in field 9E has lasted for more than a quarter of a century (Borneman and Becker, 2007). The objective of the current project was to determine if similar sites with suppressiveness against *H. schachtii* could be detected in southern California where host crops of the nematode were grown. In a bioassay of field soils, reproduction of introduced *H. schachtii* in infested non-treated and infested methyl iodide-fumigated portions of the soils were compared and used as an indication of *H. schachtii* suppressiveness. Results from the population density assay were compared with those of a cyst-baiting assay that determined fungal parasitism of nematode eggs (Sikora *et al*., 1994).

MATERIALS AND METHODS

A total of fifty-nine soil samples were collected from agricultural fields located in southern California (primarily Imperial and Santa Barbara Counties). Sampling areas cropped to *H. schachtii* host plants were chosen preferentially (Table 1). A previously described *H. schachtii*-suppressive soil from Riverside County (Westphal and Becker, 1999) and a conducive sandy river bottom soil were included as controls. Soil texture compositions were analyzed by a commercial laboratory (A&L Western Agricultural Laboratories,

Modesto, CA). Soil samples for the greenhouse experiments were composites of multiple subsamples from the top soil layer (atleast to a depth of 10-15 cm) that were used after screening (6-mm screen) and mixing 10:1 (w/w) with steam-pasteurized silica sand for improved drainage and aeration. Each soil was divided into two portions; one was non-treated and the other was fumigated with methyl iodide (MI) (500 kg ha^{-1}) (Becker et al., 1998). Initially, a subset of eleven soils was collected and processed to establish, which of these contained soil suppressiveness to *H. schachtii* with a standard method (Westphal and Becker, 1999). To simplify the experimental procedures, a second bioassay based on counting the number of white females on host roots observable through a transparent planting pot wall was tested. Once the usefulness of the second method was confirmed in comparison to the development of cyst population densities of *H. schachtii* in larger pots, another 48 soils were evaluated. The same set of soils was analyzed for its egg-parasitic potential (Sikora *et al*., 1994) with an additional short-term bioassay. For all soils, population densities of *H. schachtii* were determined at initiation and harvest of the greenhouse assays by extracting nematode cysts from a 350-g subsample from each pot using a modified Fenwick flotation can (Caswell *et al*., 1985) with an efficacy of ca 80% from moist sandy loam soil (Westphal and Becker, 1999). Nematode cysts and eggs were counted with a dissecting microscope.

Bioassay with second-stage juveniles (J2) of H. schachtii (initial test)

Non-treated and MI-fumigated portions (1.6 kg each) of eleven field soils were placed in 12-cm clay pots and planted with 7-week-old seedlings of Swiss chard, *Beta vulgaris* 'Large White Ribbed' (Lockhart Seeds Inc. Stockton, CA). The plants were arranged in a greenhouse in a randomized complete block design with five replicates and were incubated at ambient light and a temperature of 24 ± 3°C. Two weeks after planting, each pot was infested with 3,000 J2 of *H. schachtii.* The plants were watered with a low volume drip irrigation system (Netafim Irrigation Inc., Fresno, CA) and fertilized at weekly intervals with 50 ml of a nutrient solution (2.64 g L^{-1} of Miracle Gro with 15% N, 13.2% P, 12.5% K, Scotts Co., Marysville, OH). Eleven weeks after infestation, the plant tops were excised at soil level. Cysts and soil were dislodged by shaking and washing off the roots and then returned to the bulk of the pot contents before cysts and eggs were extracted for counting as previously described. The root oven-dry weights were determined.

Table 1. Origin and soil texture classes of the 59 soils used in this study for population suppressiveness against *Heterodera schachtii*.[a]

	Number of soils per county[b]	
Soil texture class	Santa Maria	Imperïal
Clay	4	23
Clay loam	2 (1)	1
Sandy clay	0	1
Silty clay loam	2	0
Sandy clay loam	8	1
Loam	8 (2)	0
Sandy loam	7	0
Sum of Soils	31	26

[a]Soils were collected from representative areas of southern California fields; plus one sandy loam (Riverside County) and one sandy soil (river bottom).
[b]The numbers in parentheses indicate the detected frequency of suppressive soils within the specific soil class.

Bioassay with cysts of H. schachtii

Cyst infestations were used for an initial bioassay with the eleven soils that had been previously conducted with J2 infestation as well as with the remaining 48 field soil samples. Non-treated and MI-fumigated portions of test soils were individually mixed 2:1 (w/w) with *H. schachtii*-infested sandy soil from greenhouse cultures adding ca. 70 eggs per g^{-1} soil (contained within cysts) for the bioassay and ca. 100 eggs per g^{-1} soil in the survey test of 48 soils. In both experiments, soil mixes were distributed into 250-ml transparent polystyrene cups, and adjusted to 17.6% soil moisture. Each treatment was replicated five times. The cups were closed with a polyethylene lid and incubated at 24 ± 3°C in the dark. After one month, the lids were removed, drainage holes applied at the bottom of the containers, and the cups wrapped with aluminum foil. All cups were seeded with four surface-disinfected (five minutes in 20% commercial bleach solution, 5.25% NaOCl) seeds of mustard-greens, *Brassica juncea*, 'Florida Broadleaf' (Lockhart Seeds Inc., Stockton, CA) and arranged in a randomized complete block design in the greenhouse at 24 ± 3°C and ambient light. After emergence, the seedlings were thinned to one plant per pot. The plants were watered as needed, and fertilized as described for the first experiment. Eleven weeks after seeding, the aluminum foil was removed from the pots, and the white *H. schachtii* females visible through the translucent polystyrene cup wall were counted. The cups were

wrapped with aluminum foil again and incubated in the greenhouse for an additional two weeks. At termination of the experiment, cysts and eggs of *H. schachtii* were extracted and enumerated. The roots were oven-dried and weighed.

Assay for egg-pathogenic fungi

The potential for fungal infections of nematode eggs in the various survey soils was assessed by the method of Sikora *et al*. (1994). Fifteen mature cysts of *H. schachtii* of equal size, obtained from greenhouse cultures, were placed onto a sheet of nylon gauze (100 µm diam pores) that was folded and inserted into a plastic slide frame (Pacon Plastic Slide Mounts, Pakon, Inc. Minnetonka, MN). The frames were closed and buried in 6.7 × 6.7 × 7.2 cm (L × W × H) plastic pots (Anderson DIE and MFG Co., Portland, Oregon) each containing ca. 300 g of the 48 field test soil-silica mixes (10:1; V:V). The pots were arranged in a greenhouse at 24 ± 3°C in a randomized complete block design with five replicates. The soils were kept moist during the 14 days of incubation. The frames were removed from the soil and the cysts from each frame were collected with tweezers. The cysts of each replicate were crushed in a tissue homogeniser (Glass Col, Terre Haute, IN) and the released eggs were collected on a sieve of 25 µm aperture. A 0.5% Rose-Bengal (Matheson Company, Norwood, OH) solution (diluted in 5% ethanol) was added to the egg suspension (1:1) and incubated overnight at 4°C to stain the fungal hyphae growing from the egg as well as infected juveniles in the eggs. The suspension was decanted onto a sieve of 25-µm aperture, rinsed with demineralized water, and transferred into a counting chamber. The eggs were observed at 100X with a microscope, and the extent of infection was estimated from a total of two subsamples of 100 randomly chosen eggs each. An egg was counted as infected when fungal growth was detected within its shell.

Data analysis

All data were subjected to statistical analysis with SuperANOVA (Abacus Concepts, Inc., Berkeley, CA). In the initial bioassay, treatments were compared by contrast at $P \leq 0.10$. In the more extensive survey, data of non-treated or MI-fumigated portions for each soil origin were log-transformed [$\log_{10}(x+1)$] and compared by F-test at $P \leq 0.10$. For both tests with transparent containers, soils with at least one significant difference between non-treated and fumigated portions were selected. The relationship of number of females observed through the transparent wall of the growth containers and number of cysts extracted from the container soil were tested by regression analysis using PROC REG in SAS (SAS 9.2.1; SAS Institute, Cary, NC). A linear or quadratic regression was used to fit the model to the data of each experiment. After comparison of the linear slopes, a single regression line and the 95% confidence intervals were illustrated.

RESULTS

Bioassay with second-stage juveniles (J2) of H. schachtii (initial test)

In these two experiments with the eleven soils, soils 4 and 10 were consistently suppressive to the *H. schachtii.* More detailed observations were as follows. It was aimed at identifying the most appropriate method to detect suppressive soil.

Bioassay with J2 of H. schachtii

In one soil (soil 8), significantly more *H. schachtii* cysts g^{-1} soil were observed in the fumigated than in the non-treated soil when the soils were planted to Swiss chard, infested with *H. schachtii* juveniles (Table 2). Four soils (4, 8, 9, 10) had significantly higher numbers of eggs/cyst in the fumigated than the non-treated portions at harvest (Table 2). In the other soils, the nematode population densities between non-treated and MI-fumigated portions of each soil were not different. Significant differences in root dry weights between plants from non-treated and MI-fumigated portions were observed only in four soils (Table 2).

Bioassay with cysts of H. schachtii

Soil 3 had more cysts/g of soil in the fumigated, re-infested than the non-treated, infested portion (Table 3). The numbers of eggs $cyst^{-1}$ were higher in the fumigated portions in soil 3, 4 and 10 in comparison to the non-treated portions of these soils (Table 3). The numbers of females were significantly different in non-treated and fumigated portions of soil 5 and 11 (Table 3). Root dry weights in the MI-fumigated soil 6 and 10 were significantly increased compared to the non-treated equivalents (Table 3).

Survey of California cropping areas

Only data of the sixteen of the 48 soils that exhibited significant differences in *H. schachtii* population development or plant growth data between the re-infested fumigated and non-fumigated portions are shown (Table 4). These were soils of diverse soil textures and different initial population densities (Table 4). Two of these soils supported a higher *H. schachtii* reproduction after MI-fumigation in comparison to the non-treated equivalent (soils 24, 25; Table 4). This higher reproduction was measurable in the number of females per plant, number of eggs/cyst and in one of the two soils in the number of cysts/g of soil (Table 4). Both soils were from agricultural fields near Santa Maria, CA.

Table 2. Soil texture, origin, and population densities of *Heterodera schachtii* in non-fumigated (NF) or fumigated (500 kg methyl iodide/ha; F) California soils, infested with 3,000 J2/pot and cropped for 11 weeks to Swiss chard 'Large White Ribbed'.[a]

Soil				Root dry weight [g]			Cysts/g soil			Eggs/cyst		
No.	Texture[b]	Origin[c]	Pi[d]	NF	F	*P*	NF	F	*P*	NF	F	*P*
1	C	IV	395	0.19	1.04	0.0038	0.72	0.72	N.S.	123.49	175.51	N.S.
2	C	IV	38	1.12	0.84	N.S.	0.64	0.75	N.S.	139.75	201.02	N.S.
3	C	IV	48	0.98	1.03	N.S.	0.93	0.99	N.S.	111.59	143.47	N.S.
4	Cl	SM	571	0.88	0.91	N.S.	0.66	1.16	N.S.	61.10	176.17	0.0220
5	Scl	SM	486	1.25	0.77	N.S.	0.920	0.93	N.S.	75.84	134.99	N.S.
6	Scl	IV	52	0.82	1.05	N.S.	0.90	1.02	N.S.	88.34	124.75	N.S.
7	L	SM	357	1.89	0.83	0.0212	1.00	1.31	N.S.	107.96	179.74	N.S.
8	Sl	SM	957	0.98	1.32	N.S.	0.59	1.03	0.0688	41.90	128.28	0.0287
9	Sl	SM	667	0.26	1.54	0.0415	0.45	0.91	N.S.	11.95	123.12	0.0207
10[e]	Sl	RI	1224	0.10	0.60	N.S.	0.39	0.79	N.S.	17.32	127.46	0.0825
11	S	RB	N/A	1.08	0.60	0.0943	0.66	0.73	N.S.	142.52	159.02	N.S.

[a]Values of non-fumigated (NF) and fumigated (F) soil portions were compared by F-test. The probability (*P*) was indicated: differences at $P \leq 0.10$ were noted, comparisons at $P > 0.10$ were considered non-significant (N.S.).
[b]Soil texture classes were determined after texture analysis and assignment to the US soil texture class system: C: clay; Cl: clay loam; Scl: sandy clay loam; L: loam; Sl: sandy loam; S: sand.
[c]Soils were collected from: IV Imperial: Valley; SM: Santa Maria; RI: Riverside; RB: River bottom.
[d]Initial population (Pi) of eggs per 100 g of soil were determined by standard procedures (Caswell *et al.*, 1985).
[e]Soil 10 was from field 9E at the agricultural research station of the University of California, and had been found previously to be suppressive to *H. schachtii* (Westphal and Becker, 1999).

Table 3. Root dry weight, number of females on root surface observed through transparent pot wall, and population densities of *Heterodera schachtii* in some non-fumigated (NF) or pre-inoculation-fumigated (500 kg methyl iodide/h; F) soils, infested with 70 eggs/g of soil and cropped for 11 (13) weeks to mustard-greens 'Florida Broadleaf".[a]

Soil	Root dry weight [g][b]			Females[c]			Cysts/g soil[b]			Eggs/cyst[b]		
No.	NF	F	*P*	NF	F	*P*	NF	F	*P*	NF	F	*P*
1	2.68	3.55	N.S.	5.20	17.60	N.S.	3.98	4.61	N.S.	124.32	157.97	N.S.
2	3.56	3.37	N.S.	27.20	37.60	N.S.	5.15	4.75	N.S.	181.78	192.19	N.S.
3	2.11	2.96	N.S.	28.40	36.80	N.S.	3.47	5.32	0.0037	94.71	180.05	0.0908
4	1.20	1.82	N.S.	1.20	16.20	N.S.	3.43	4.55	N.S.	25.42	176.36	0.0120
5	3.11	2.73	N.S.	21.60	57.00	0.0832	5.19	4.61	N.S.	122.11	128.33	N.S.
6	2.24	4.05	0.0176	19.60	12.80	N.S.	3.63	3.35	N.S.	124.79	125.13	N.S.
7	2.04	3.24	N.S.	78.20	62.80	N.S.	4.81	4.84	N.S.	117.71	136.81	N.S.
8	1.02	1.64	N.S.	0.60	2.40	N.S.	2.77	2.78	N.S.	11.95	42.00	N.S.
9	1.43	1.40	N.S.	0.20	5.60	N.S.	2.74	2.72	N.S.	9.87	11.68	N.S.
10[d]	0.92	2.48	0.0498	0.00	2.25	N.S.	2.62	3.08	N.S.	9.43	83.43	0.0029
11	2.20	2.83	N.S.	14.20	1.80	0.0442	2.85	2.66	N.S.	35.69	40.55	N.S.

[a]Values of non-fumigated (NF) and fumigated (F) soil portions were compared by F-test after log-transformation [$\log_{10}(x+1)$]. The probability (*P*) was indicated: differences at $P \leq 0.10$ were noted, comparisons at $P > 0.10$ were considered non-significant (N.S.).
[b]At harvest after 13 weeks, plant measures were taken and nematode cysts and eggs extracted for counting.
[c]Females visible through transparent pot walls were counted after eleven weeks.
[d]Soil 10 was from field 9E at the agricultural research station of the University of California, and had been found previously to be suppressive to *H. schachtii* (Westphal and Becker, 1999).

Table 4. Soil texture, origin, egg-pathogenic potential (EPP), and population densities of *Heterodera schachtii* in selected non-fumigated (NF) or pre-inoculation-fumigated (500 kg/ha methyl iodide; F) soils, infested with 100 eggs/g soil and cropped for 11 (13) weeks to mustard-greens 'Florida Broadleaf'.[a]

	Soil				Root dry weight			Females/container[b]			Cysts/g soil			Eggs/cyst		
No.	Text[c]	Orig[d]	Pi[e]	EPP[f]	NF	F	*P*	NF	F	*P*	NF	F	*P*	NF	F	*P*
12	C	IV	143	8.7	2.42	2.79	N.S.	6.25	19.50	0.0407	3.50	3.36	N.S.	57.40	103.77	N.S.
13	C	IV	5	0.8	3.23	2.64	N.S.	29.80	21.20	N.S.	4.18	2.80	0.0041	161.94	96.71	N.S.
14	C	IV	10	1.8	2.93	3.57	N.S.	8.50	14.25	N.S.	4.12	3.03	0.0676	97.34	106.91	N.S.
15	C	IV	5	8.4	2.68	2.83	N.S.	6.75	18.20	0.0732	3.16	3.15	N.S.	103.27	67.02	N.S.
16	Cl	IV	14	6.2	3.38	3.58	N.S.	14.40	4.60	N.S.	3.08	3.10	N.S.	95.14	36.68	0.0833
17	Sicl	SM	619	2.2	1.60	1.67	N.S.	5.00	15.00	0.0900	2.46	3.12	N.S.	28.65	38.55	N.S.
18	Sicl	SM	210	1.5	1.76	2.34	N.S.	3.00	23.80	0.0768	2.86	3.44	N.S.	29.16	55.18	N.S.
19	Scl	SM	5	7.7	1.82	3.45	N.S.	37.00	19.60	N.S.	3.74	2.93	N.S.	132.36	38.56	0.0039
20	Scl	SM	2638	1.0	2.36	1.08	0.0258	12.00	14.75	N.S.	2.67	2.67	N.S.	18.53	10.23	N.S.
21	Scl	SM	14	3.7	2.72	1.59	0.0516	10.00	17.60	N.S.	2.83	3.56	N.S.	51.00	20.84	N.S.
22	Scl	SM	700	3.2	2.19	2.76	N.S.	2.80	2.50	N.S.	2.67	2.49	N.S.	28.31	15.25	0.0772
23	Scl	SM	76	6.0	0.99	2.78	0.0436	4.00	8.25	N.S.	2.90	2.94	N.S.	38.61	17.98	N.S.
24	L	SM	671	2.7	1.91	1.82	N.S.	2.80	7.50	0.0766	2.56	3.06	0.0497	6.62	32.84	0.0938
25	L	SM	143	0.8	1.38	2.08	N.S.	1.80	7.20	0.0872	3.01	3.03	N.S.	10.65	34.28	0.0603
26	Sl	SM	214	5.8	1.05	1.22	N.S.	3.50	2.00	N.S.	2.27	2.20	N.S.	22.03	3.75	0.0001
27	Sl	SM	105	19.1	3.38	2.70	N.S.	52.80	13.50	N.S.	3.68	2.36	N.S.	156.44	24.26	0.0332

[a]Treatments followed by N.S. were not significantly different when tested with *F*-test at $P < 0.10$; females were counted after eleven weeks, all other measures were taken after 13 weeks of greenhouse incubation. Only those soils that showed significant differences in at least one parameter are presented.
[b]Females were counted through the transparent pot wall of a 250-ml container.
[c]Soil texture classes were determined after texture analysis and assignment to the US soil texture class system: C: clay; Cl: clay loam; Sicl: silty clay loam; Scl: sandy clay loam; L: loam; Sl: sandy loam; S: sand.
[d]Soils were collected from: IV: Imperial Valley; SM: Santa Maria.
[e]Initial populations (Pi) of the eggs per 100 g of soil were determined by standard procedures (Caswell *et al*., 1985).
[f]Fungal infection of the eggs was assessed as percentage of two samples each of 100 randomly chosen eggs after staining according to Sikora *et al*. (1994).

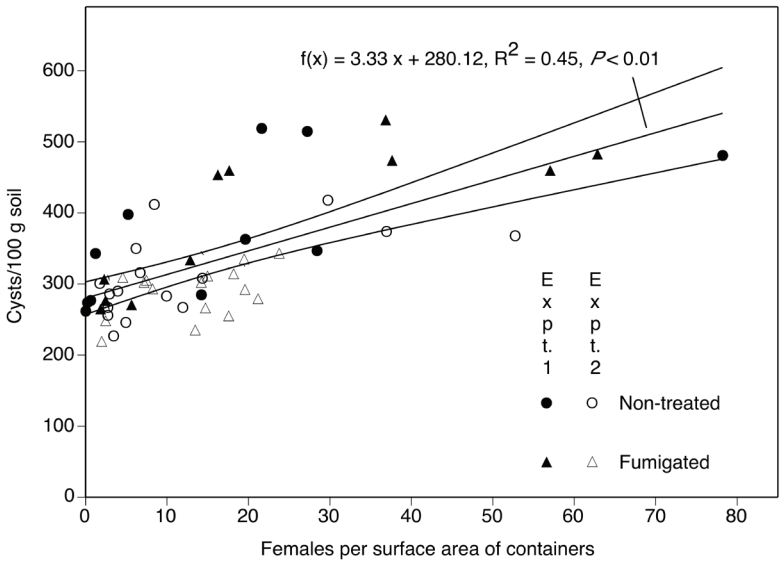

Fig. 1. Number of cysts 100 g^{-1} soil compared to females visible through the transparent wall of 250-ml containers filled with *H. schachtii*-infested soil that was non-treated or fumigated with methyl iodide. Data were derived from eleven (Expt. 1) and sixteen soils (Expt. 2) in a greenhouse assay. The mutual linear regression line with the 95% confidence intervals is given.

Seven soils supported higher nematode reproduction in the non-treated than in the MI-fumigated soil after infestation with *H. schachtii* (soils 13, 14, 16, 19, 22, 26, 27; Table 4). These differences were detected in the abundance of cysts/g of soil (soils 13 and 14) or the number of eggs/cyst (soils 16, 19, 22, 26, 27; Table 4). In these soils, there were no differences detected in the number of females when comparing non-treated with MI-fumigated portions (Table 4).

In six soils (soils 12, 15, 17, 18, 24, 25, Table 4), more females were detected in the re-infested MI-fumigated portions of the soils than in the non-treated equivalents (Table 4). The number of cysts/g of soil and the number of eggs/cyst were not different in these comparisons, with the exception of soils 24 and 25 (Table 4). In the remaining 32 soils, there were no differences detected in nematode reproduction in comparing infested, non-treated with infested, MI-fumigated portions of each soil (data not shown).

Female nematode counts through the transparent container surfaces were correlated with the number of extractable cysts 100/g of soil at harvest of the growth containers in both experiments (Expt. 1: $f(x) = 3.15 x + 317.15$; $R^2 = 0.48$; $P < 0.01$; Expt. 2: $f(x) = 2.38 x + 269.34$; $R^2 = 0.28$; $P < 0.01$). The slopes were not significantly different ($P_{Experiment \times Females} = 0.4531$), therefore data were illustrated with a mutual regression line and the corresponding confidence intervals at $P = 0.05$ (Fig. 1).

Egg-pathogenic potential

Fungal egg infection ranged from 0 to 19.1 %. The infection frequencies of the eggs in the assay and the numbers of eggs/100 g of soil at collection of the field soils expressed no quantitative relationship (data not shown). Frequencies of fungal infection of the eggs and the number of eggs/cyst at harvest of the greenhouse assay were weakly correlated ($R^2 = 0.21$; $P < 0.05$; data not shown).

DISCUSSION

Biological suppressiveness to *H. schachtii* was found in less than 7% of the soils examined. Soils with various soil texture classes (one clay loam, two loam, and one sandy loam soil) exhibited soil suppressiveness against *H. schachtii*, suggesting that suppressiveness was not directly correlated with soil texture. In the more extensive survey with 48 soils, soil fumigation led to increased *H. schachtii* population density development in three soils in addition to the previously studied soil 9E after reinfestation (Westphal and Becker, 1999). The cyst nematode population increase was similar to the one observed in earlier experiments in the *H. schachtii*-suppressive 9E soil. This soil and one soil from Santa Maria (soil 4) of the initial bioassay set of eleven soils consistently supported lower *H. schachtii* population development in the respective non-treated

portions versus MI-fumigated portions. In addition, two loam soils were identified as suppressive in the more extensive survey. This low frequency of detectable suppressive soils in a variety of soil textures suggests that beneficial soil suppressiveness can occur in various edaphons but that the natural population density-regulation may not be sufficiently supported in the current California production systems.

In a previous study of the distribution of soil suppressiveness against the sugar beet cyst nematode, resident *H. schachtii* cysts were extracted from the test soil, squashed through a screen, and amended as a slurry into conducive soil (Nicolay and Sikora, 1989). That method hypothetically introduced egg parasites into the conducive soil, and according to the frequencies of the parasites, different population developments were expected. However, only a slight negative correlation was detected between number of egg parasites in the soil and *H. schachtii* population development (Nicolay and Sikora, 1989). This lack of relationship of the frequencies of egg parasites and population development in greenhouse culture was confirmed in our current study. The egg pathogenic potential did not allow prediction of final population parameters of the greenhouse assay; responses appeared random. This suggests that more than a single mode of action, e.g., based on egg parasitism, could potentially suppress cyst nematodes.

In this project, the duration of the greenhouse incubation combined with the host cropping for two nematode generations soil-exposed life stages of *H. schachtii* came in contact with the soil environment. The comparison of introduced nematode population development in the non-treated versus respective fumigated soil samples was appropriate for detecting differences in *H. schachtii* receptivity in a larger number of field soils. Soil fumigation was followed by changes in root mass of bioassay plants; such changes of plant growth often challenge experiments with field soils in greenhouse pots (Westphal and Becker, 1999). In the current tests, feeding site availability was assumed to be of limited importance for difference in nematode reproduction. The test had its challenges in greenhouse maintenance. For example, watering was difficult for the various soil texture soils, and other methods for the detection of soil suppressiveness against cyst nematodes (Borneman and Becker, 2007; Westphal, 2005) may enable overcoming these problems.

Female nematodes enumerated in these experiments through the transparent container walls were correlated with cyst population densities determined by extraction and enumeration. Because the female numbers predicted less than half of the variability of the cyst numbers they were not reliable to indicate exact quantitative nematode population development. This lack of correlation is a common problem when only surfaces of growth containers are used for nematode population density estimates (Hirling, 1970; Müller *et al.*, 1990). However, the female counts would have decreased the number of samples required for processing if only soils with more females in MI-fumigated than in non-treated portions were extracted. Extracting and counting of the *H. schachtii* cysts and their eggs remained the most reliable method for determining nematode population densities.

Surprisingly, some soils displayed a lower nematode multiplication after fumigation and consequent *H. schachtii*-infestation than in the equivalent non-treated infested portions. The number of endemic initial populations of *H. schachtii* had very little impact on final population densities in the greenhouse assay. This was apparent in the initial bioassay assay where the suppressive standard soil had the highest endemic population densities but yet did not allow for reproduction in the non-treated soil portions. In the extensive survey test, a higher nematode population occurred in the non-treated portion than in the fumigated, reinfested part only in soils that had very low initial population densities that accounted for ≤ 0.2 % of the total egg population density at initiation of the greenhouse test. Although the mechanisms of this reproduction is not understood we can only assume an extremely high reproduction in these field soils.

In summary, the data illustrate that the comparison of population development of *H. schachtii* in non-treated and MI-fumigated portions of field soils has the potential to detect suppressiveness in multiple soil texture classes. More importantly, soil suppressiveness existed in various soil texture classes, suggesting the broad potential for directly exploiting the natural mechanisms that reduce population densities of soil-borne plant-parasitic nematodes for sustainable agricultural production.

ACKNOWLEDGEMENTS

Parts of these studies were supported by the Sustainable Agriculture Research and Education Program (SAREP), the Storkan-Hanes-Research Foundation and the German Academic Exchange Service (DAAD). The authors thank E.T. Natwick (UCCE Imperial County) and Craig Reade (Betteravia Farms, Santa Maria, CA) for locating the soil sampling sites, C. Adams and E. Moll for discussion of the statistical analysis. The authors thank J. Darsow for technical assistance.

LITERATURE CITED

Baker, K. F., and R. J. Cook. 1974. Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN.

Becker, J. O., H. D. Ohr, N. M. Grech, M. E. McGiffen, Jr., and J. J. Sims. 1998. Evaluation of methyl

iodide as a soil fumigant in container and small field plot studies. Pesticide Science 52:58-62.

Borneman, J., and J. O. Becker. 2007. Identifying microorganisms involved in specific pathogen suppression in soil. Annual Review of Phytopathology 45:153-172.

Caswell, E. P., I. J. Thomason, and H. E. McKinney. 1985. Extraction of cysts and eggs of *Heterodera schachtii* from soil with an assessment of extraction efficiency. Journal of Nematology 17:337-340.

Chen, S. Y. 2007a. Suppression of *Heterodera glycines* in soils from fields with long-term soybean monoculture. Biocontrol Science and Technology 17:125-134.

Chen, S. Y. 2007b. Tillage and crop sequence effects on *Heterodera glycines* and soybean yields. Agronomy Journal 99:797–807.

Crump, D. H. 1989. Interaction of cyst nematodes with their natural antagonists. Aspects of Applied Biology 22: Roots and the Soil Environment, 135-139.

Crump, D.H., and B. R. Kerry. 1987. Studies on the population dynamics and fungal parasitism of *Heterodera schachtii* in soil from sugar-beet monoculture. Crop Protection 6:49-55.

Garrett, S. D. 1955. A century of root-disease investigation. Annals of Applied Biology 42:211-219.

Gray, N. F. 1985. Ecology of nematophagous fungi: Effect of soil moisture, organic matter, pH and nematode density on distribution. Soil Biology and Biochemistry 17:499-507.

Hirling, W. 1970. Untersuchung von Bodenproben auf Kartoffelnematoden (*Heterodera rostochiensis* Woll.) mit der Sieb-Schlämm-Methode und dem Biotest nach Behringer. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 77:498-505.

Jaffee, B. A. 2000. Augmentation of soil with the nematophagous fungi *Hirsutella rhossiliensis* and *Arthrobotrys haptotyla*. Phytopathology 90:498-504.

Jones, F. G. W. 1975. The soil as an environment for plant parasitic nematodes. Annals of Applied Biology 79:113-139.

Kerry, B. R. 1987. Biological control. Pp. 233-263 *in* R. H. Brown and B. R. Kerry, Eds. Principles and practice of nematode control in crops. Sydney, Australia: Academic Press..

Kerry, B. R., and D. H. Crump. 1980. Two fungi parasitic on females of cyst-nematodes (*Heterodera* spp.). Transactions of the British Mycological Society 74:119-125.

Kerry, B. R., D. H. Crump, and L. A. Mullen. 1980. Parasitic fungi, soil moisture and multiplication of the cereal cyst nematode, *Heterodera avenae.* Nematologica 26:57-68.

Linford, M. B., F. Yap, and J. M. Oliveira. 1938. Reduction of soil populations of the root-knot nematode during decomposition of organic matter. Soil Science 45:127-141.

Mueller, J., W. Steudel, and J. Schlang. 1990. Vergleich von Extraktionsverfahren und Biotest zur Bestimmung von Populationsdichten des Rübennematoden (*Heterodera schachtii)*. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 97:449-457.

Nicolay, R., and R. A. Sikora. 1989. Techniques to determine the activity of fungal egg parasites of *Heterodera schachtii* in field soil. Revue de Nématologie 12:97-102.

Norton, D. C. 1979. Relationship of physical and chemical factors to populations of plant parasitic nematodes. Annual Review of Phytopathology 17:279-299.

Rhoades, H. L. 1980. Reproduction of *Belonolaimus longicaudatus* in treated and non-treated muck soil. Nematropica 10:139-140.

Sikora, R. A. 1992. Management of the antagonistic potential in agricultural ecosystems for the biological control of plant parasitic nematodes. Annual Review of Phytopathology 30:245-270.

Sikora, R. A., R.-P. Schuster, and S. Kiewnick. 1994. Indexing biodiversity and antagonistic potential in agricultural soil of Madagascar. Mededelingen Faculteit Landbouwwetenschappen Universiteit Gent 59:781-790.

Stirling, G. R. 1991. Biological control of plant parasitic nematodes: progress, problems and prospects. CAB International, Wallingford, UK, 282 pp.

Stirling, G. R., and L. J. Smith. 1998. Field tests of formulated products containing either *Verticillium chlamydosporium* or *Arthrobotrys datyloides* for biological control of root-knot nematodes. Biological Control 11:231-239.

Stotzky, G., and R. T. Martin. 1963. Soil mineralogy in relation to the spread of Fusarium wilt of banana in Central America. Plant and Soil 18:317-337.

Stotzky, G., and L. T. Rem. 1966. Influence of clay minerals on microorganisms. I. Montmorillonite and kaolinite on bacteria. Canadian Journal of Microbiology 12:547-563.

Westphal, A. 2005. Detection and description of soils with specific nematode suppressiveness. Journal of Nematology 37:121-130.

Westphal, A., and J. O. Becker. 1999. Biological suppression and natural population decline of *Heterodera schachtii* in a California field. Phytopathology 89:434-440.

Xing, L. J., and A. Westphal. 2006a. Reduced severity of sudden death syndrome and low population densities of *Heterodera glycines* in a soybean monoculture soil. Phytopathology 96:S125 [abstract].

Xing, L. J., and A. Westphal. 2006b. Transferring soil suppressiveness against *Heterodera glycines* under

field conditions. Journal of Nematology 38:302 [abstract].

Xing, L. J., and A. Westphal. 2009. Effects of crop rotation of soybean with corn on severity of sudden death syndrome and population densities of *Heterodera glycines* in naturally infested soil. Field Crops Research 112:107-117.

Received: *20/X/2010*

Recibido:

Accepted for publication: *20/IV/2011*

Aceptado para publicación: